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BILHARZIOSIS IN SOUTH AFRICA.

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A FEW years ago it was believed that there was only one form of trematode responsible for the pathological conditions included in the term Bilharziosis, namely a worm, known as *Schistosoma haematobium*, which has had numerous other synonyms, but as I am dealing with the subject purely from a South African point of view, I need merely mention that given it by Dr John Harley, namely *Distoma capense*.

It is now recognised that there are several varieties of Bilharzial parasites.

In South Africa, I have only found evidences of *Schistosomum haematobium* in both its vesical and intestinal manifestations. *Schistosomum japonicum* has not come under my notice.

In this country the disease caused by these parasites is an important one, especially among the native races; far more important than I think is generally believed. So many medical men, born in this country, have in their youth passed blood-stained urine, or have been accustomed to hear of friends doing so, without suffering apparent serious consequences, that they are liable to look upon it as an affection of the bladder only, and to attach little importance to it, forgetting possibly that it may attack other organs, frequently with serious consequences.

In many parts of the Cape Colony and the Transvaal, a large number of the European youths contract the disease. School boys in these parts have competitions as to who can pass a urine of the deepest colour, and the boy most seriously infected is considered more fortunate than his fellows.

Doubtless most of the European boys in Cape Colony, Natal and the Transvaal get rid of all symptoms at about 20 years of age, the disease dying out, possibly because the patient is removed to some

place where re-infection cannot occur. But among the natives living in the tropical or semi-tropical parts, the complaint does not take such a favourable course.

There is probably no disease which causes the native inhabitants of the Province of Mozambique so much worry and anxiety as Bilharziosis. They attribute every evil under the sun to it: impotency of men and sterility in women, are often considered the result of infection. I must admit there seems some justification for the belief. There cannot be any doubt that the disease in any case has a debilitating effect on the native's constitution, probably rendering him more subject to tuberculosis and other diseases when he leaves his kraal to seek work in a colder climate. When the parasite attacks the intestine the result is often fatal.

Geographical Distribution in South Africa.

In 1851, Bilharz and Griesinger discovered that the haematuria in Egypt was due to the presence of a trematode worm and its ova in various parts of the body. In 1864, Dr John Harley announced the discovery of the parasite in Port Elizabeth and Uitenhage (Cape Colony) and renamed the parasite *Distoma capense*. In 1888, Dr Chute, of Kingwilliamstown, wrote an important article on the subject, connecting it with the Buffalo River. Dr Darley Hartley, when practising in Kingwilliamstown and Cathcart, also had many cases of the disease, traced to bathing in this same river. It is well known that it occurs in many other places in the Colony. Dr Saunders, of Grahamstown, mentions Keiskama, Alice, Konap, Fort Beaufort, as being villages from which he has received patients who were suffering from the complaint. Several natives suffering from the parasite told me they lived near and bathed in the St John's River in Pondoland. In Pietermaritzburg (Natal), European youths have been known to be extensively infected for many years. Dr Brock (1893, *Journ. Pathol. and Bacteriol.* vol. II. p. 52) has demonstrated the prevalence of the disease in the Rustenberg district (Transvaal), especially along the slopes of the Magaliesberg and Pilansberg mountains, and in the valley of the Eland, Hex, Magalies, and Crocodile Rivers. More recently, Dr Stock, Asst. M.O.H., Johannesburg, reported on an outbreak occurring among freshly imported English troops who had bathed in the Aapies river at Pretoria. The same author states that cases have been traced from the Orange River, Vaal River, Mnkanda (Newcastle, Natal), Mooi

River, Klip River, Umzindusi (Maritzburg), and streams in Middelburg (Cape Colony and Transvaal). In 1907, Capt. Gatt, R.A.M.C., contributed an article on the occurrence of the disease among European troops infected in Middelburg (Transvaal), and since then, Dr Arnold has reported that numbers of the civil inhabitants of Middelburg, who have bathed in either the Groot or Klein Oliphants River, have contracted the complaint. It is said that in the Klein Oliphants River there is one particular pool in which it is considered to be especially dangerous to bathe.

A statement has been made that people do not contract Bilharziosis in those rivers running west. This statement is borne out to some extent by references to Table I, which gives the result of microscopical examinations of samples of urines taken from natives of various tribes. It may be noticed that in no case were any Damaras or British Basutos found to harbour the parasites. Further, an educated British Basuto, employed in the hospital, tells me that he has never heard of people in his country passing blood with their urine.

The foregoing information collected from various sources should, with Table I, which gives results of urine examinations, and Table II, giving the number of times the parasites were found in livers at autopsies, make the geographical distribution fairly clear. These tables should also assist one in forming an estimate of the percentage of natives infected in each tribe, but a more accurate idea on this point can be obtained by referring to Table III, under the heading of Parasites.

Besides these:—

Triodontophorus deminutus was found on 1 occasion,
Physaloptera mordens „ „ 2 occasions
Trichocephalus trichiurus „ „ 2 „

We see therefore that about 33·5 % of natives have the *Bilharzia* parasite in the liver. The finding of the parasite in that position is often a matter of difficulty, consequently it must be admitted that any conclusions drawn from these figures will err on the small side.

The figures showing the numbers of other parasites found demonstrate the extent to which the South African native is infected. We may therefore consider the disease to be spread over the following areas. Commencing at the north, Nyassa, Mozambique and Quilimane natives are largely infected, that is to say, the people inhabiting the East Coast country between the southern border of German East Africa and

the Zambesi. South of the Zambesi, we find that natives recruited near Beira, on the banks of the Pungwe river, carry the parasite. In the area inland to this strip of coast country, we see in the Table II that a certain percentage of the British Central African natives, those from the eastern border of Lake Nyassa, the Angonis from the banks of the Zambesi, the Makalanga from Matabeleland, have the disease. On the coast line, south of latitude 22° S. are the Myambaams, Mtyopis, Shangaans and Lourenco-Marques boys; each of these tribes has a certain number of their people infected. That is to say, those people living between the Big Sabi river and Lourenco Marques. Inland of these coast countries, we have Swaziland and the Transvaal. Table I shows that some of the Swazis have the disease, and we know from reports concerning the European population that many parts of the Transvaal are grossly contaminated. The number of sick natives

TABLE I.

Showing the results of microscopical examination for Bilharzia ova of a number of urines collected from various tribes of natives.

Tribe	Number examined	Positive results	Percentage
Mozambique	190	48	25·8
Quilimane*	60	3	5
Lake Nyassa	22	8	36·3
British Central African	80	7	8·7
Shangaan	32	6	18·7
Mixed East Coast gang of Shangaan, Mtyopi, Myambaam, and Lourenco-Marques boys ...	225	26	11·5
Zulu	34	8	23·5
Pondo	10	2	20
Swazi	44	8	18·1
Mxosa	39	2	5·1
Transvaal Basuto†	59	1	1·6
British Basuto	68	—	0·0
Makalanga	45	10	22·2
Damara	50	—	0·0
Bechuana	12	2	16·6
Total	970	131	14·0

* The results obtained from the examination of urines taken from these natives are interesting. The boys were not fresh recruits; they had all been working on the mines for 12 months. The ova in those urines giving positive results were few and far between. Post-mortem examination of fresh recruits show a very much higher per cent. infected. See Table III. This seems to suggest the advantage of removing patients from an infected area.

† The positive result obtained among the Transvaal Basuto was in urine from a boy who had been living in the Rustenburg district for some time.

TABLE II.

Showing the number of times the Bilharzia worms were found at autopsies held on natives of various tribes. Also the number of times with which intestinal parasites were present.

Tribe	No. examined	Bilharzia worms in liver	Ankylostomes	Round worms	Tape worms
Mozambique	215	102	139	17	1
Quilimane	129	60	81	32	1
Nyassa	52	16	27	10	2
British Central African	10	4	2	1	1
Beira	3	1	2	0	0
Angoni	14	2	3	1	0
Shangaan	65	23	32	27	2
Tonga	6	0	1	1	2
Myambaam	106	28	30	36	7
Mtyopi	40	9	15	8	8
Transvaal Basuto	12	1	1	0	1
Damara	19	0	2	1	0
Swazi	1	0	0	0	1
Pondo	6	0	0	1	3
Mxosa	5	0	0	2	1
Mixed Races	9	0	0	0	0
Total	692	246	335	137	30

from the Transvaal coming under my care is not so large as from other parts of South Africa. I have not therefore personally much evidence on the subject, but a medical missionary, Dr Liegme, in a paper he read before the Society for Advancement of Science in South Africa, states that haematuria is frequent in the Spelonken. Table I demonstrates the Zulu to be infected, and we know that the European population in parts of Natal likewise have the disease. I have had several cases among Amapondo and Amaxosa natives of Cape Colony, and as I have already stated the disease is prevalent in many places in the Eastern Province. Of the Western Province, I cannot speak definitely. British Basutoland has, so far, produced no patients for me. Damaraland is also free as far as I can judge by examining recruits from that country. Of the Orange River Colony, I am not in a position to speak decidedly, but I think one may anticipate that a certain percentage of the population on the north-eastern border will be infected.

Source of Infection.

That the disease is conveyed by some means from infected water there is no doubt, but except that the miracidium is killed by minute traces of hydrochloric acid not a single experimental fact has yet been brought to light indicating the actual source and mode of infection. The old idea in Cape Colony, still adhered to curiously by some, was that the organism in some form crept up the urethra while the persons were bathing. For this reason some of the inhabitants of Cape Colony used to tie their genital organs up in a handkerchief before going into a river to bathe. This custom may have induced some writers erroneously to try and prove that the prepuce cover worn by certain South African tribes was originally adopted as a protection against the parasite.

That infection occurs in some manner while bathing I think there can be little doubt. Dr Darley Hartley, when practising in East London and Cathcart, saw many cases of Bilharziosis. They always occurred in boys or young adults, and he tells me that in almost every case he obtained a history of them having bathed in the Buffalo River.

Dr Brock (1893, *Journ. Pathol. and Bacteriol.* vol. II. p. 52), relates an exactly similar experience when he lived in the Rustenburg district (Transvaal). Dr J. Allen (1888, *Practitioner*, vol. XI. p. 310) at one time Medical Officer for the Corporation of Maritzburg (Natal), writes as follows: "Nearly all the youths bathing in the Umzimdusi and Dorp Spruit are infected, while the girls, who do not bathe, remain free of the disease." In the two outbreaks among English troops in the Transvaal, the men infected had been bathing in spruits or rivers. The only case of a European girl becoming infected, that I can find notes of, is one reported by Dr C. P. Childe (III. 1899, *Brit. Med. Journ.* p. 644), and this girl, it was found on enquiry, had been in the habit of bathing in a freshwater pool on a farm in Natal. The facts seem to support the theory that infection occurs through the skin, for if the disease is contracted through drinking water, the girls would be infected as well as the boys, as, though the girls rarely bathe, they, in most cases drink the same water.

Moreover, when travelling among the natives on the East Coast, where both sexes drink the same water, and both bathe to about the same extent, I noticed that the women seemed, if anything, more commonly infected than the men; certainly the most severe types of the disease occurred among females. I was constantly requested to give medicines for women who were passing blood in their urine.

Dr Brock says that persons bathing in the streams skirting Rustenburg complain of severe itching when emerging from the water. Dr John Harley noticed that people in Cape Colony often suffered from nodular excrescences of the skin, which later became transformed into indolent ulcers. He considered these consequent upon the invasion of the parasites into the skin when bathing. Personally I have not come across any skin eruptions which in any way suggest the entrance of any kind of embryos or larvae, unless perhaps a native skin disease known as Dwappo.

I think the preceding statements make it far more probable that infection does not occur by the drinking water, but rather through the skin while a person is wading or washing in infected rivers or pools, the worm entering in the same manner as the larvae of the *Ankylostoma duodenale*.

It has been urged against the probability of infection occurring through the skin while bathing, that monkeys contract the disease, and these animals certainly do not bathe, while they constantly drink water from streams or pools when they cannot get dew, and in doing so, probably immerse their feet at the edge of the water. It seems to me most probable that the disease is contracted either by bathing or wading in infected water or mud.

I may add here that Europeans have noted that certain pools in the rivers are more fruitful in producing cases of the disease than others. The natives on the East Coast state that some cases are produced by bathing in certain pools, but they also state that it is caused by drinking M'Jumbula spirit (a filthy spirit made by the distillation of a preparation of manioca root)!

Cobbold originally suggested that there must be an intermediate host in the life history of the parasite, but to the discussion of this subject I am not in a position to add any fresh information.

Incubation Period.

Dr Stock in his article on *Bilharzia*, published in the *Lancet* of September 29th, 1906, quotes the cases under Dr Abercrombie's charge which occurred among newly arrived drafts of young soldiers who bathed in supposedly infected pool near Pretoria. Of these, the shortest time in which symptoms developed after bathing was one month, the longest two months. He accordingly concluded that the incubation period lasted about six weeks.

In connection with this it must be remembered that people can probably carry the parasites for some considerable time without any signs of the disease being apparent, provided they lead a quiet life, but the symptoms may at any time appear suddenly in consequence of some severe exertion. That the period of incubation according to Stock is shorter than that observed by others may be due to the fact that the troops in question were cavalry, subject to rough riding; a form of exercise most likely to bring on the symptoms at an early date.

Symptoms.

Before discussing this portion of the subject, I must state that a number of my patients spoke a language understood by no Europeans that I know, and by very few natives. Consequently, to obtain any account as to how the patient really felt was difficult and to obtain anything approaching an accurate history of the cases was impossible. But the symptoms I noted do not appear to vary much from those reported, as occurring among the inhabitants of other countries. It is therefore only necessary to discuss them briefly.

They appear to vary in severity in different localities. Consider for example the case of European and native youths in parts of the Transvaal, Natal and Cape Colony, where, though numbers are infected, the majority only have a certain amount of haematuria; they may at times show evidences of debility, but they are not often really seriously incapacitated. On the other hand, among the natives on the East Coast and more tropical parts, there is, as stated before, probably no disease which causes the inhabitants so much worry as Bilharziosis.

This may probably be due to there being more than one species of parasite; the population of the southern parts are only infected with the variety of disease which attacks the urinary system; that of northern and tropical parts harbouring both kinds. At the same time, it is also possible that the infection in the northern districts may be more extensive than in the southern.

The individual symptoms may be divided into those affecting (1) the urinary system; (2) the intestines.

Urinary System.

Haematuria is the most prominent and important symptom of them all.

It occasionally appears suddenly; the result of an accident. I had

a patient, a young Pondo, who told me he first noticed that he was passing blood in his urine after a fall from a horse.

In some cases, though the urine appears quite clear, if it is treated in a centrifuge, and then examined under a microscope, red blood corpuscles and a few ova will be found. At other times, the whole urine passed is faintly blood-stained, or the first water evacuated may be quite clear, a few drops of pure blood only appearing at the end of micturition. With some cases, small tube-shaped blood clots are passed in the urine stream. In the most severe cases, almost pure blood is emitted and occasionally this may coagulate in the bladder so firmly as to cause complete suppression of urine. I have only seen the last condition once; it was a case of a European who came from the Rustenburg district.

With severe haematuria cystitis often develops, thus necessitating frequent micturition, which, with the anaemia and the mental worry, has a very debilitating effect on the system. I must, however, admit that I have never had a native or European die of uncomplicated Bilharzia disease of the bladder, though I have seen some excessively severe haemorrhages as a result of it.

The haematuria is frequently brought on by violent exertion, especially riding.

On several occasions, I have had patients admitted to hospital who rolled on the floor in intense pain, which they referred to the pubic region; they had apparently no other symptom, though one native had incontinence of urine. With a catheter a small amount of purulent urine, containing *Bilharzia* ova, was drawn off. When I first saw these patients I thought they had received an irritating poison, and that possibly some native doctor had been administering a mixture somewhat more searching in its effects even than those they usually employ. However, hot fomentations, hypodermic injections of morphia and urotropine administered by the mouth, relieved the symptoms, and after a few days the patients recovered sufficiently to be transferred to the convalescent room. They remained fairly well there for a week or two. After this time, they were readmitted to hospital with indefinite symptoms and often died suddenly within a few days. At other times, these patients lay wrapped in their blankets in a drowsy condition for days and weeks. Beyond the fact that their urine contained albumen, which is so common an occurrence among native patients, that one has to disregard its presence, there were no symptoms indicating the cause of the condition. The temperature on admission usually being above

normal, but within a few hours falling to subnormal and remaining so until death.

A post-mortem examination usually showed that all the organs except those of the urinary system were healthy. But the kidneys were in a state of profound hydronephrosis, the organs being mere shells.

Urinary Fistula.

In accounts of the disease by Egyptian observers fistula is noted as a common complication.

The number of natives I see suffering with fistula is small. I have never seen one which I suspected was due to Bilharziosis. But most of my patients are adult males in the prime of life; the condition may be more frequent among the old men, unfit and unlikely to seek for work away from their own country, though when I have been in the kraals I have not noticed an abnormal number of perineal complaints.

Of the many complications which must occur when the parasites attack the genito-urinary organs of the female I have had no experience.

Intestinal Bilharziosis.

The symptoms of this condition in my experience are exceedingly indefinite; they are most liable to be mistaken for those of chronic diarrhoea or dysentery.

Those cases I have diagnosed during life have been identified more by the exclusion of other diseases than anything else. Because though I make microscopical examinations of the faeces, the number of ova found in the specimens often did not convey any correct impression as to the extent of the disease. It seems that the ova really causing the mischief are those working in the tissues. One may even, in severe cases of Bilharzial infection of the intestine, experience a little difficulty during life in demonstrating the ova, though after death a scraping from the mucous membrane of the rectum shows the tissues to be infiltrated with them.

This peculiarity of the disease accounts for it not being more often recognised when examinations of faeces are made say for the ova of *Ankylostomum duodenale*. When I see a patient suffering from chronic diarrhoea passing five to six motions daily without much slime or blood, and not responding to ordinary treatment, but wasting and becoming anaemic to a degree quite out of proportion to the extent of

the diarrhoea, I suspect Bilharziosis. I do not wish to infer that my patients never pass blood or slime in their motions but, as a general rule, they have not done so; their motions recall those of a patient with acute tuberculosis, complicated with diarrhoea.

Straining and tenesmus have not been marked features of the disease, though one patient was in agony for several days before death unless under the influence of morphia. I particularly lay stress on the absence of these symptoms, because I note in other countries they are reported as being marked features of the disease. It may be interesting to note here that, though I have experienced some most virulent epidemics of dysentery among natives, attended by a very heavy mortality, tenesmus, which is most frequently a very trying feature of the disease when Europeans are attacked, was comparatively uncommon among my coloured patients.

Temperature.

The temperature in intestinal Bilharziosis cases under my notice has been, as a rule, normal or subnormal, varied at intervals of seven or eight days by a two or three days irregularity. This disturbance may have been of malarial origin; at the same time I suggest it may have been due to a crop of embryos or miracidia having burst from the ova into the tissues.

Duration of the Disease.

In Cape Colony and more southern parts of South Africa one generally expects the symptoms to abate and finally disappear when the patient reaches to about twenty years of age. But that the disease may persist for a long time without any re-infection occurring is proved by Maj. Freeman (III. 1907, *Journ. Roy. Army Corps*, vol. VIII. p. 278) who quotes the case of a soldier who was infected in Pretoria and afterwards returned to England. Three years after his return, living ova could still be found in his urine. I am inclined to think it doubtful whether a person, once he has contracted the disease, and the walls of his bladder and the surrounding tissues have become impregnated thoroughly with calcified ova, will ever be completely free of all evidences of the complaint. I think that years after all active mischief has ceased, scraping of the bladder tissue would still show calcified ova, or their débris. That is to say, the ova can be demonstrated long after the actual trematode producing them has been destroyed and absorbed.

The Parasites.

I do not intend to enter into the anatomical description of the actual parasites or to give an account of the dimensions or other minute details of their ova. That is a matter to my mind requiring the knowledge of an expert helminthologist.

The natives of South Africa are infected then with the two forms of the disease, viz. the classic form and Manson's Bilharziosis.

I have found both terminal and lateral spined ova at autopsies and when examining the excreta from patients in hospital, and I believe the parasites which deposit these two distinct forms of ova normally infest different organs, though the terminal spined ova occasionally trespass on the preserves of the lateral spined one.

I have been able to find the worms most readily in the portal veins of the liver (see Table II), but the parasites in that situation are, I understand from Dr Leiper (London School of Tropical Medicine), to whom specimens were submitted, of the male sex and immature. To find the mature worms it is necessary to examine the ramifications of the venous system of the bladder and rectum. I have never found worms or ova in the arterial system.

Some of the ova probably remain in a calcified state long after the worms have disappeared from the system. That there may be two separate trematodes is, I think, supported by the fact that the terminal spined ova is the only variety ever found by me in the urine or mucous membrane of the bladder and ureters[1].

In the faeces and mucous membrane of the large intestine lateral spined ova are generally found, with sometimes, however, a few terminal spined ones amongst them, and these latter are frequently in a calcified state, suggesting that they have worked their way from the bladder tissues rather than that they have been deposited by the blood stream. On four occasions at autopsy (examinations on two Angonis, one Shangaan, and one Quilimane native) I found lateral spined ova in the mucous membrane of the large intestine, but although I carefully scraped and examined every portion of the mucous membrane of the bladders, no ova of any kind could be found in these organs.

My observations of the occurrence of the lateral spined ova are

[1] In a discussion reported in the *Lancet* of April 20th, 1889, Dr Moon stated that "he had found both terminal and lateral spined ova in both bladder and rectum." This statement does not hold good in South Africa.

confined to what is known as the low veldt in South Africa. I have not found lateral spined ova in natives coming from other than tropical or semi-tropical parts. There is too the marked difference in the severity of the general symptoms of the disease in the two localities.

To demonstrate approximately the number of times the terminal spined ova were produced as compared with the lateral spined ones, and also to ascertain in what proportion of cases both kinds of ova could be found in the same person, I made a careful microscopical examination of scrapings taken from the mucous membrane of the bladders and large intestines obtained at 90 consecutive autopsies. These examinations were conducted with special care, and I think the results given in the following Table III may be relied on absolutely.

TABLE III.

Showing results of microscopical examination of scrapings taken from the mucous membrane of bladders and large intestines at 90 consecutive autopsies; also showing the number of times the Bilharzia worm was found in the vessels of the liver.

		Bladder			Large intestine				Liver	
Tribe	Total no. of examinations	Terminal spined ova	Lateral spined ova	Not microscopically examined*	Lateral spined only	Terminal spined only	Mixed lateral and terminal spined ova	Not microscopically examined	Worms present	Not found
Myambaam	11	10	0	0	2	1	2	0	3	8
Mtyopi	5	5	0	0	3	0	0	0	1	4
Shangaan	8	7	0	0	2	1	0	1	2	6
Nyassa	3	3	0	0	0	0	0	0	1	2
Mozambique	30	24	0	2	3	5	3	4	10	20
Quilimane	15	12	0	0	4	0	2	2	4	11
Angoni	15	11	0	2	4	1	2	3	3	12
Transvaal Basuto	3	2	0	0	0	0	0	0	0	3
Total	90	74	0	4	18	8	9	10	24	66

* A few separate organs escaped microscopical examination for unavoidable reasons; the numbers so escaping have been noted in a special column.

In four of the above cases, namely, two Angonis, one Shangaan, and one Quilimane native, lateral spined ova were found in the mucous membrane of intestine but no ova could be found in the bladder. In only nine cases was the Bilharzia ovum absent in all organs. One Mozambique native gave negative results in the bladder and intestine but a single *Bilharzia* worm was found in the liver. The numbers of terminal spined ova found in mucous membrane of the large intestine were usually very few.

To Table III, I may add that I found the terminal spined ova in the following organs:

(1) Bladder.
(2) Ureters.
(3) Kidneys.
(4) Rectum.
(5) Veins of foregoing Organs.
(6) Prostate Gland.
(7) Mesenteric Gland.
(8) Lungs.

I have not examined the heart or pancreas for ova, and on only two or three occasions, the spleen substance. I examined the urethra on several occasions, always with negative results.

The lateral spined ova I found principally in the rectum and occasionally a few in the liver. It is interesting to note that I have not found the terminal spined ova in this last organ, though I expected to do so.

The above facts seem to point to a wide area of location of the terminal spined ovum, but a much more restricted field for lateral spined ova.

The Ova.

The following is a rough description of the two forms of ova, as I have seen them, which may be of interest, though it does not enter into minute details.

Terminal Spined Ova.

These may be observed in three forms:—

(*a*) The light coloured animated ovum with the clear well marked contents.

(*b*) The ovum with homogeneous black contents, which are apparently calcified, evidently dead and incapable of doing any more damage to the tissues than coarse pigment granules would do.

(*c*) Shells of ova from which the miracidia or embryos have escaped.

Those ova found outside the urinary system or intestines were, to the best of my recollection, all of them in the calcified condition described under heading (*b*).

Lateral Spined Ova.

These may also be seen in several forms:—

(*a*) The light coloured animated ovum with the prominent lateral spine.

(*b*) The light coloured animated ovum, with a spine looking like a thin thorn projecting from the surface.

(*c*) The ovum with a very thick spine, producing the appearance almost of a division of the lower end of the egg. This represents, I believe, the calcified state in the terminal spined ovum.

(*d*) Shells of the lateral spined ova from which the embryos have escaped.

As regards the first two forms of the lateral spined form, it must be noted that at times, on first looking at them, it appeared as though they had no spines at all. But immediately on pressure being brought to bear on them through the cover-slip, a spine shoots out. I think this is in most cases an optical delusion, and that what really happens is that the ova were lying with the spines on their inferior surface, and that pressure on the cover-slip causes the eggs to turn over, so bringing the spine suddenly into view.

I may mention that I frequently, when examining scrapings from the mucous membrane of the bladders and intestines, found empty ova shells, from which the miracidia or embryos had escaped. In Cobbold's *Entozoa*, it stated that Griesinger found a number of empty eggs in the left ventricle of the heart. Other authorities have doubtless observed the same fact. I have also, when examining perfectly fresh specimens, especially those taken from the mucous membrane of the rectum, observed that the slightest pressure has caused the ova to burst and the field of the microscope has been suddenly populated by numbers of rapidly moving miracidia. These statements suggest to my mind that the ova do occasionally dehisce while still within the human system, and explain occasional evidences of recrudescence of the disease.

Pathological Anatomy.

The changes caused by this parasite are principally to be seen in the urinary and alimentary systems.

(1) *Urinary System.*

Calculus. I am dealing with this subject separately, as I consider my observations are of especial interest. I notice that in Egypt the frequency with which natives suffer from stone in the bladder is ascribed to Bilharziosis. In South Africa, of the 66 autopsies I have made on natives infected with the disease, in which the bladder has been particularly examined, I have not once found a stone. Further, I

have never had a patient presenting the clinical symptoms of calculus of the bladder. In view of Egyptian records this experience appears extraordinary.

Bladder. This organ is the one most commonly affected. As stated before, I have only made four autopsies on natives found to harbour the parasite, in which the bladder escaped infection. The effect of the ova on the coats of the organ varies greatly, according to the numbers present, and to the length of time they have had to produce the changes.

In some the only feature noticeable on first opening the bladder is a small slightly inflamed spot on its mucous membrane; in others, one is at once struck by the presence of several acutely congested papilliform bodies projecting into its interior, which would undoubtedly bleed when squeezed by the contraction of the bladder at the end of micturition. In more advanced cases still, the area of acute congestion is extensive, affecting nearly the whole of the internal surface, in the centre of which there may be a patch of black gangrenous mucous membrane, easily detachable. Such a condition might result in a very large haemorrhage. It would appear that the varying degrees of inflammation described above represent the sub-acute and acute stage of the disease, and that at a later date, when the parasite dies out, or the ova for some reason lose their activity, other changes occur, which can be looked upon as representing a more or less chronic condition.

I make this statement, because, in other bladders I have found on careful examination faintly coloured brownish spots on the mucous membrane, not gritty to the touch, or raised above the surface, in which a few terminal spined calcified ova can be found on microscopical examination. This condition is, I believe, the chronic stage of the sub-acute condition described first, in which there was only very slight congestion.

Following this stage a little further, one finds bladders in which there are four to five greyish-brown patches, of about 15 mm. in diameter, slightly raised above the surface of the mucous membrane; these when scraped with a scalpel can be heard and be felt to be gritty. Later still the spots are much more elevated, and have blackened tops, which project into the bladder, and resemble warts which have been burned with caustic. In other bladders, nearly the whole extent of the internal surface is affected. Such bladders when opened and spread out, resemble a sheet of very fine sandpaper, and on the surface of some of them I have seen small pin-head vesicles, which are hard, feeling like

small nodules of cartilage. They have an opalescent appearance, glistening in bright sunlight. If one of these vesicles be removed and placed under a cover-slip it takes a certain amount of pressure to burst it. Microscopically, it consists of a capsule containing a little clear or cloudy fluid, in which is suspended a nest of black terminal spined ova, or the débris of ova. I think it may be stated that the various coats of the bladder become thickened to a greater or less degree in every case of the disease (due to the interstitial growth of fibrous tissue. See Bowley, *Lancet*, April, 1889). In some, the thickening is very slightly marked, but in others they may be half an inch thick, like the walls of a uterus. Such a condition greatly reduces the capacity of the organ, and at the same time diminishes its power of expansion.

Ureters. These are the organs which, next to the bladder, are most commonly affected. The portions of the ureters which pass through the bladder wall, and the first two inches of their lower ends, are the parts usually implicated. Occasionally patches of infection of about an inch in extent, may be found midway between the bladder and the kidneys, sometimes even nearer than this to the latter organs. The conditions of the mucous membrane of these tubes vary markedly. In some the affected parts have a soft pale cream-coloured velvety appearance. In others there are dark brown gritty patches. When such occur near the bladder, the lumen of the tube is frequently distended, and almost blocked with masses of brown sandy material looking somewhat like coffee grounds. On one occasion I removed about half a tea-spoonful of this substance from each ureter, which microscopically I found to consist of black terminal spined ova and their débris.

Once I found the mucous membrane sprinkled with dark specks like black powder grains. These were exceedingly hard. I broke a cover-slip in crushing one of them. I believe they consisted of calcified débris of ova, and that they are a secondary condition to the little vesicles already described as appearing in the bladder, as I found similar vesicles in the ureters in close proximity to the black specks. On one occasion the lumen of the left ureter was completely obliterated in four places, and the kidney on the corresponding side was cystic and atrophied. With adherent tissues it only weighed 37 grammes.

The walls of the ureters become hypertrophied in the same manner as do those of the kidneys.

Kidneys. Calculus. On only one occasion have I found a stone in the kidney. It was about the size of a millet seed and lay in the calyx of the left organ.

Though I have constantly traced the Bilharzia ova from the bladder up the ureters to hilum of the kidney, it would seem that it is comparatively rare for the kidneys themselves to be affected by the disease in its acute stage, the ova not being found in the substance of these organs in large numbers. But the kidney is sometimes markedly affected secondarily to the pathological changes in the bladder and ureters. There are three main causes inducing kidney changes: (1) as the coats of the bladder become thickened they tend to cause a partial occlusion of the lumen of the ureters passing between them; (2) the deposit of coffee-ground material already described, at the lower end of the ureters, reduces their lumen; (3) the thickening of the coats of the ureters has the same effect.

These three conditions retard the urine from entering the bladder, and set up a back pressure, which eventually causes hydronephrosis and later pyonephrosis, in some cases to such a degree that after death the kidneys are found to be mere shells. The following table shows the number of times, in 420 autopsies, in which there were cysts in the kidneys or the calices were dilated.

TABLE IV.

Tribe	Number examined	Cystic kidney		Per cent.
Nyassa	36	0		0·0
Mozambique	160	15		9·4
Quilimane	56	6		10·7
Beira	2	0		0·0
Myambaam	64	7		10·9
Mtyopi	24	0		0·0
Shangaan	38	1		2·6
Tonga	2	0		0·0
Angoni	15	1		6·7
Mashona	1	0		0·0
Basuto	7	1		14·2
Mxosa and Pondo	5	0		0·0
Damara	10	1		10·0
Total	420	32	or	7·6

I have seen it stated that the kidneys of persons affected with Bilharziosis weigh more than the normal. This, one would naturally expect to be the case, but my limited figures do not bear the statement out, as, out of five cases which I proved microscopically did not contain the ova in the bladder, the average weight of the two kidneys was 284·4 grammes, whereas the average weight per pair of fifty pairs of kidneys

taken from bodies proved microscopically to have the ova in the bladder was only 270·75 grammes. The figures are too few to place much reliance on them, but they strike one as peculiar. Of the other parts connected with the genito-urinary system, I shall refer to:

Prostate gland. I have frequently found a few nests of terminal spined ova in this gland. I have not seen any marked naked-eye lesion in it, and my attention has not been called to it clinically in any way.

Urethra. Though I have examined the lining membrane of this tube microscopically on several occasions, I have never found it to be affected. But I note that in other countries the urethra is sometimes attacked.

Foreskin. On several occasions I noticed a hypertrophied condition of the foreskin, which I suspected to be caused by Bilharzial infection but I have never confirmed the diagnosis.

Alimentary canal. The large intestine is the portion of the alimentary canal most frequently affected. In some cases the mucous membrane of the lower bowel is acutely congested, without any evidence of ulceration. At other times the whole surface seems honey-combed with clear punched-out ulcers of about the size of a split pea. In others again, there is a granulating condition, reminding one of the surface of the skin following a burn, in that state which, as a student, I was taught should be rubbed with bluestone before any attempt should be made to skin graft. At a later stage to this a papillomatous condition is found, reminding one of haemorrhoids.

Besides these acute conditions a stage is often found, which I believe corresponds to the chronic sandy stage in the urinary system—black leaden-coloured patches of mucous membrane without ulceration, a deep scraping from which shows black lateral spined ova. In some, this pigmented state may be so marked that the whole surface of the mucous membrane looks like a strip of black velvet. This peculiar colouration has always been associated with lateral spined ova. As in the bladder, so in the intestines the various coats are greatly hypertrophied.

I have not looked for the disease in other parts of the alimentary tract, but Dr May, the Government Bacteriologist, examined a cyst from the stomach of an imported Chinaman, which contained terminal spined ova.

Liver.

The numbers of South African natives who contract a cirrhotic condition of the liver is, I think, large. In the following table, No. V, we see that out of 739 post-mortems, the liver was found to be cirrhotic on 106 occasions, or 14·3 %.

TABLE V.

Table showing the number of times in which at 739 autopsies the liver was found to be cirrhotic.

Tribe	No. of examinations	Cirrhotic liver		Percentage
Nyassa	53	5		9·4
Mozambique	221	27		12·2
Quilimane	132	29		21·9
Myambaam	114	21		18·4
Mtyopi	44	7		15·9
Shangaan	74	10		13·5
Tonga	6	1		16·6
British Central Africa	15	3		20·0
Matabele	1	0		0·0
Makalanga	4	1		25·0
Transvaal Basuto	11	1		9·1
Zulu	1	0		0·0
Mangwato	2	0		0·0
Rhodesian	2	0		0·0
Beira	2	1		50·0
Angoni	15	0		0·0
Mashona	1	0		0·0
British Basuto	2	0		0·0
Pondo	8	0		0·0
Mxosa	4	0		0·0
Fingo	1	0		0·0
Damara	26	0		0·0
Total	739	106	or	14·3

Whether this cirrhosis is entirely due to *Bilharzia* is, I think, doubtful, as, though when we analyse the Table, we see that the tribes with the highest percentages affected with cirrhotic livers come from those areas where the lateral spined ovum is most prevalent, yet it must not be forgotten that these are also the very districts where malarial fever exists in its worst forms, and where, above all, alcohol is consumed by the natives in enormous quantities. In the Portuguese Territories natives can obtain as much imported spirit as they can pay for. Furthermore, numbers of them are adepts at the distillation of

spirits. Provided with a couple of kaffir pots and an old gun barrel, the native soon starts a very efficient distillery. The country abounds with vegetable products from which he can make spirits, the two commonest of which are, I think, the Cashew apple and the Manioca root. In consequence of the ease with which a native can obtain alcohol, he consumes, as stated, enormous quantities, and I think it may truly be said that, in certain seasons of the year, he is never properly sober.

If we consider the other liver complaints from which the native suffers, viz. waxy disease or fatty degeneration, we find them to be above the normal, as shown in the following Table:

TABLE VI.

Table showing number of times the liver was found to be either waxy or fatty at 400 *autopsies.*

Tribe	Number examined	Waxy	Fatty
Shangaan	38	4	3
Myambaam	64	4	3
Mtyopi	24	3	0
Nyassa	36	1	1
Mozambique	180	2	6
Quilimane	56	1	3
Beira	2	1	0
Total	400	16	16

I do not think that it has been suggested that Bilharziosis is responsible for either of these conditions.

The trematode itself is not found most frequently in the cirrhotic livers, as, out of a hundred consecutive autopsies, fourteen showed a cirrhotic condition of the liver, and in these the worms could only be found twice, whereas they were found twenty-four times in the remaining livers, which were either normal or merely congested. This feature may be due to the fact that the cirrhotic liver has often very little blood in it, consequently it is difficult to express the worms. And it is also possible that the worms only stay in the liver when in an immature state, therefore not laying eggs; that at maturity they seek other spheres, and the eggs then laid are carried back to the liver and set up the cirrhotic condition.

I am not in a position to speak too definitely on this matter, but the number of ova found in the liver tissues does not appear to be sufficient to warrant the conclusion that they are responsible for the cirrhosis.

It is generally accepted that the ova, and not the worms, cause the pathological changes.

Mesenteric Glands.

These glands in a patient with intestinal Bilharziosis are slightly enlarged: they are of a very dark colour and of hard consistence. I have frequently found terminal spined ova when examining scrapings taken from them.

Lungs.

Of the few lungs I have examined I have only found the ova on one occasion (terminal spined ova) in a boy who died from acute pulmonary tuberculosis. The tuberculosis was evidently secondary to the Bilharzial infection. It is reasonable to suppose that a Bilharzial infection of the lungs would render the patient more susceptible to infection with *Bacillus tuberculosis*. It is interesting to note that Cobbold suggests the peculiar susceptibility of the South African native to pneumonia may be the result of Bilharzia.

Treatment.

I have only heard of three native remedies, but doubtless many others exist: (1) A decoction made from the roots of a *Cassia*, growing in Equatorial East Africa, which Dr O'Sullivan Beare obtained from the natives of those parts. This plant has since been named *Cassia beareana*; an extract made from the roots has been used with marked success in the treatment of Blackwater fever (see O'Sullivan, *Lancet* of Feb. 1st, 1902, and Bostock, *Transvaal Med. Journ.* of July, 1907). (2) While among the kraals in the Inhambane District, I was informed by natives that they used a medicine, consisting of a decoction of the leaves of a tree called *Umrangala Umgubo* (spelt phonetically). The use of this plant for bladder troubles would appear very widespread, as a Mozambique boy told me that a decoction of the roots of the same plant was used in his country by persons passing blood in their urine. Among the Mozambiques, he informed me, the plant is known by the name of *Reba Reba*: I regret to say that I have not been able to get this plant identified. (3) A demulcent drink made from the beard of mealie cobs has a great reputation for the treatment of the disease among some natives.

While suffering from an attack of the disease natives in Portuguese Territory abstain from eating Cashew apple (*Anacardium occidentale*) and from drinking wine or spirit manufactured from it. As stated previously, some natives attribute the disease itself to the drinking of manioca spirit.

Küchenmeister's Manual suggests the use of onions and garlic in the treatment of the disease. Griesinger prescribed a mixture of calomel and turpentine.

Methylene blue has a reputation in some parts. Liquid extract of male-fern in 10 m. doses, twice daily, has its advocates. Santonin, quinine, cubebs, sandalwood oil, benzoic acid, have all been recommended.

Personally I have obtained the best results in the treatment of Bilharziosis affecting the bladder by the use of urotropine, 5 gr. doses night and morning, in half a pint of water, with rest in bed. Should the haemorrhage become severe I use adrenalin. With this treatment I have always been able to quiet the symptoms, but I am inclined to think that I have been singularly fortunate in the class of patient with which I have had to deal.

As regards the treatment of the intestinal disease, I have to admit that I know of no drug which has the slightest influence on it. I have used infusion of monsonia the most frequently, after that magnesium sulphate, opium, astringent remedies and other drugs. I may almost say that I have worked steadily and conscientiously through the British *Pharmacopoeia* without any satisfactory result.

Prognosis.

This varies according to the system of the body which is infected. If it is purely an uncomplicated case of terminal spined ova affecting the bladder of a male, the consequences are not as a rule very serious in South Africa. If, on the other hand, the disease has obtained a firm hold of the mucous membrane of the large intestine, I consider the prognosis of the case a grave one.

NOTE ON SPIROCHAETES IN CASTRATION TUMOURS OF PIGS.

By J. BURTON CLELAND,

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(*From the Pathological Laboratory, Department of Public Health.*)

In pigs in Western Australia it is of frequent occurrence to find at the seat of castration large oval fibrous tumours from the size of an hen's egg to that of a tennis ball. These have a thick fibrous wall with a cavity in the centre, which is frequently small in relation to the size of the mass. The walls of this cavity are brownish-yellow and degenerated and the contents usually sero-pus of a similar colour, though at times a large quantity of ordinary whitish purulent matter is found. In films made from this brownish-yellow pus and stained with weak carbol-fuchsin or Leishman's stain, varying numbers of spirochaetes mixed with minute cocci and bacilli and larger, occasionally spore-bearing, organisms will be found. These spirochaetes vary in length from 6 μ or less to 12 μ; their thickness varies from the most delicate tenuity to that of a tubercle bacillus; the spirals may be three or four and perfectly regular or, on the other hand, quite irregular, and acute bendings even at a right angle may be seen. Further, some of the large apparently bacillary organisms may show slight undulations suggesting that they are large forms of this spirochaete. Sections of the tumour show a fibrous stroma, becoming more cellular towards the centre where it passes into necrosed tissue swarming with organisms, amongst which, sometimes in masses, spirochaetes may be found. Where the still living cells abut on the necrosed area, a varying number of eosinophile cells are revealed by Leishman's stain.

The occurrence of this spirochaete is especially interesting when viewed in connection with the presence in man under certain conditions of *Spirochaeta refringens*. Whether the spirochaete found in the

tumours has a definite etiological relation to their formation or is only an accidental denizen of their cavity, introduced at the time of castration with the organism causing the swelling, it is impossible to say.

Recently Sydney Dodd, in the *Journal of Comparative Pathology* vol. XIX. 1906 (vide note, *Journal of Tropical Vet. Science*, vol. II. no. 2), has described an ulcerative skin disease of pigs, in which the ulcers contained spirochaetes apparently somewhat similar to these.

NOTES ON THE DRUG TREATMENT OF CANINE PIROPLASMOSIS.

By GEORGE H. F. NUTTALL, M.A., M.D., Ph.D., Sc.D., F.R.S. and G. S. GRAHAM-SMITH, M.A., M.D.

The observations relating to the treatment of canine piroplasmosis by means of drugs are usually very briefly recorded so that the precise conditions under which they were conducted cannot be ascertained.

In South Africa Hutcheon (1893, p. 477, and 1899, p. 400) recommended the use of repeated doses of ammonium chloride and belladonna, a form of treatment tried by Borthwick at Port Elizabeth with "excellent results." Subsequently Hutcheon obtained encouraging results from the use of quinine, benzoate of soda and carbolic acid. Robertson (1901, p. 332) tried calomel, quinine, ammonium chloride, extract of belladonna, carbolic acid and benzoate of soda without success. In fact he thought that carbolic acid hastened death. He obtained the best results from a "calomel pill to start with, then a calomel and quinine pill four times a day." Without stating the dose he says that very large amounts of calomel are needed. Hutcheon, on the other hand, did not approve of the calomel treatment.

In Europe Piana and Galli-Valerio (1895) attributed the recovery in one case to the use of quinine. Almy (10. x. 1901, p. 379) treated dogs with quinine hydrobromate, but found it to be as ineffective as quinine has been shown to be in the treatment of Tristeza (piroplasmosis of cattle).

Though various other remedies have been suggested and tried, none have yet been discovered which produce any marked effect on the course of the disease. In a few cases successful results have been claimed, but importance cannot be attached to these isolated instances since dogs occasionally recover without treatment.

We have made a few carefully conducted experiments with selected drugs. In each case two dogs of about the same age and size were inoculated at the same time with similar doses from the same specimen of defibrinated heart blood obtained from a dog dying from the South

African strain of *P. canis*. One dog was kept as a control and the other treated. One experiment was made with quinine bihydrochloride, two with tartar emetic, a drug which has been used with some success in trypanosome infections, two with sodium-methyl-arsenate (combined with calomel), and two with methylene blue, drugs which are reputed to be of value in the treatment of this disease. Two experiments were also made with Beta-naphthylamine. In no case however was the course or duration of the disease influenced in any way by the treatment.

The records of our own experiments and of those of Gonder with atoxyl are given below.

Quinine bihydrochloride.

1. Dog I (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.
5. 2—3 infected corpuscles observed per field.
Treatment begun: 2·5 grains of quinine bihydrochloride dissolved in 1 c.c. of normal salt solution injected subcutaneously twice a day. A 5 grain calomel pill also given daily.
6. Treated as before, but the second dose of quinine raised to 5 grains.
7. Found dead.

Control dog (puppy) found dead on 6th day.
Result. Treated dog died on 7th day, control dog on 6th day.

Tartar emetic.

2. Dog II (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.
3. Treatment begun before the appearance of the parasites. It consisted of daily subcutaneous injections of 20 minims (1·18 c.c.) of 0·25 % tartar emetic.
8. A few parasites observed in the blood.
10. Many parasites in the blood. Haemoglobinuria.
11. Found dead.

Control dog (puppy) killed when dying on the 9th day.
Result. Treated dog died on the 11th day, and control dog on the 9th day.

3. Dog III (puppy).

Day 1. Inoculated with 4 c.c. of defibrinated heart blood.
6. Treatment begun: subcutaneous injections of 20 minims (1·18 c.c.) of a 0·5 % solution of tartar emetic given daily.
7. A few parasites observed in the blood.
9. Many parasites in the blood. Haemoglobinuria.
10. Found dead.

Control dog (puppy) died on the 10th day showing many parasites in its blood.
Result. Treated and control dog both died on the 10th day.

Sodium-methyl-arsenate.

4. Dog IV (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.
5. 2—3 infected corpuscles observed per field. Treatment begun: 2·5 grains of sodium-methyl-arsenate administered twice daily in a bread pill, together with a 5 grain calomel pill once daily.
6. The afternoon dose of arsenate raised to 5 grains.
7. 5 grains of arsenate given in the morning.
Died at mid-day; many parasites in the blood.

Control dog (puppy) found dead on the 6th day.
Result. Treated dog died on the 7th day and the control on the 6th day.

5. Dog V (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.
8. A few parasites observed in the blood. Treatment begun: 5 grains of arsenate and a 5 grain calomel pill given once daily.
9. Many parasites in the blood. Haemoglobinuria.
10. Found dead.

Control dog (puppy) showed many parasites on the 3rd day and died on the 6th day.
Result. Treated dog died on the 10th day, control dog on the 6th day.

Methylene blue.

6. Dog VI (puppy).

Day 1. Inoculated with 3 c.c. of defibrinated heart blood.
4. A few parasites observed in the blood. Treatment begun: 1 grain of methylene blue administered daily in the form of a pill.
7. Many parasites in the blood. Dog died.

Control dog (puppy) died on the 7th day with many parasites in its blood.
Result. Treated and control dogs both died on the 7th day.

7. Dog VII (puppy).

Day 1. Inoculated with 2 c.c. of defibrinated heart blood.
11. No parasites found up to this time.
12. Again inoculated with 5 c.c. of defibrinated heart blood.
19. No parasites found up to this time.
20. Reinoculated with 6 c.c. of defibrinated heart blood.
22. A few parasites observed in the blood. Treatment begun: 1 grain of methylene blue administered in the form of a pill daily.
24. Found dead with many parasites in the blood.

Result. No evidence that treatment had any effect.

Beta-naphthylamine.

8. Dog VIII (puppy).

Day 1. Inoculated with 8 c.c. of defibrinated heart blood.
7. A few parasites found in the blood. 5 c.c. of a 3 % solution of Beta-naphthylamine given subcutaneously.
8. Dog found dead.

Control dog (puppy) died with numerous parasites in its blood on the 6th day.
Result. Treated dog died 8th day and control dog on the 6th day.

9. Dog IX (puppy).

Day 1. Inoculated with 4 c.c. of defibrinated heart blood.
4. Few parasites found in the blood. Received 5 c.c. of a 3 % solution of Beta-naphthylamine subcutaneously.
5. Received 5 c.c. of 3 % solution of Beta-naphthylamine.
6. Not treated.
7. Found dead.

Control dog (puppy) died with numerous parasites in its blood on the 5th day.
Result. Treated dog died on the 7th day and the control dog on the 5th day.

Atoxyl.

In the experiments carried out by Gonder (1908, p. 301) the dogs were infected by means of intraperitoneal inoculations of blood containing *Piroplasma canis*. Three dogs served as controls while six dogs received treatment. One of the control dogs was treated after parasites appeared in its blood (control to experiments V and VI). We have not experimented with this drug but have condensed Gonder's descriptions in such a manner as to make them easily comparable with our own observations.

I. Dog "2" (1·25 years old) treated with atoxyl before inoculation. The dog received subcutaneous injections of a 1 % solution of atoxyl every two days during two weeks, the dose being gradually raised from 5 c.c. to 10 c.c. (The total amount of the drug injected was not stated.)

Day 1. Inoculated with 3 c.c. of blood.
3. A few parasites found in the blood.
4. Many parasites in the blood. Haemoglobinuria. The condition grew steadily worse and
14. the dog died of piroplasmosis.

II. Dog "3" (7 months old) treated with atoxyl before and after inoculation. It was treated in the same way as dog I receiving 3—5 c.c. of a 1 % solution of atoxyl on 6 days (45 c.c. of the solution in all).

Day 1. Inoculated with 3 c.c. of piroplasma blood.
4. Parasites found in the blood.
5. Died of heart failure, parasites found in the organs at autopsy.

Control to experiments I and II: dog (2·5 months old) inoculated with 2·5 c.c. of piroplasma blood. A few parasites found in the blood on the 3rd day; many on the 4th day, haemoglobinuria. Found dead on the 6th day.

Result. The atoxyl treated dogs died on the 14th and 5th days respectively after inoculation, the control dog on the 6th day.

III. Dog "6" (1·5 years old) treated with atoxyl before and after treatment. It received subcutaneous injections of a 1% solution of atoxyl every second day, the dose increasing gradually from 4 to 9 c.c. before the dog was inoculated. (79 c.c. of the drug were administered.)

Day 1. Inoculated with 3 c.c. of piroplasma blood.
3. Received 10 c.c. of atoxyl.
4. Parasites observed. Haemoglobinuria.
5. Many parasites in the blood. 12 c.c. of atoxyl given.
7. Received 13 c.c. of atoxyl.
9. „ 14 „
11. „ 12 „
13. „ 9 „
14. Dog died. Many parasites in the organs at autopsy. Jaundice.

IV. Dog "5" (about 6 months old) treated with atoxyl from the time it was inoculated.

Day 1. Inoculated with 3 c.c. of piroplasma blood. Received 4 c.c. of a 2% solution of atoxyl. (Total amount given during illness 13·5 c.c.)
3. Received 5 c.c. of atoxyl solution.
4. A few parasites found.
5. Many parasites in the blood. Haemoglobinuria. 4·5 c.c. of atoxyl given.
6. Dog killed, had symptoms of rabies; advanced piroplasmosis.

Control to experiments III and IV; dog (about 2 years old) inoculated with 3 c.c. of piroplasma blood. A few parasites appeared on the 3rd day and were found in the blood on the 10th day (intermittently). The dog recovered completely.

Result. Atoxyl treated dog, III, died on the 14th day, whilst dog IV was killed on the 6th day. The untreated control dog recovered completely.

V. Dog "8" (2 years old) treated with atoxyl from the time it was inoculated.

Day 1. Inoculated with 3 c.c. of piroplasma blood. Treatment: received 8 c.c. of a 1% solution of atoxyl 3 hours before inoculation.
3. Received 10 c.c. of a 1% solution of atoxyl. Many parasites in the blood. Haemoglobinuria.
6. Received 6 c.c. of a 2% solution of atoxyl.
8. Received 8 c.c. of a 2% solution of atoxyl. (Total 22 c.c. of a 2% solution.)
9. Dog killed. Heavy infection.

VI. Dog "9" (2 years old) treated with atoxyl before and after inoculation. The treatment before inoculation lasted three weeks during which period the dog received 1 % atoxyl solution in doses increasing from 2 to 12 c.c. After inoculation it received 2 % solution, the total amount given being equal to 53 c.c. of a 2 % solution.

Day 1. Inoculated with 3·5 c.c. of piroplasma blood. 14 c.c. of a 1 % atoxyl solution was given on this and the day following.
4. Received 4 c.c. of 2 % atoxyl solution.
5. Received 4 c.c. of 2 % atoxyl solution. Parasites first observed in the blood.
6. Received 4 c.c. of 2 % atoxyl solution. Parasites numerous. Haemoglobinuria.
7. Received 4·5 c.c. of 2 % atoxyl solution.
8. Fewer parasites.
9. „ „ Received 4·5 c.c. of atoxyl 2 % solution.
10. Dog very weak. Received 4 c.c. of atoxyl 2 % solution.
13. Dog too weak to stand. Received 3 c.c. of atoxyl 2 % solution.
14 and 15. Few parasites found.
16. Dog died. Death due to collapse and piroplasmosis.

Control to experiments V and VI: old dog inoculated with 4 c.c. of piroplasma blood. Parasites first found on the 4th day. This dog was afterwards treated with atoxyl every two days, the dose being raised from 4 to 8 c.c. (In all an amount equal to 70 c.c. of a 2 % solution of atoxyl was given.)

Day 5. Parasites first found. Haemoglobinuria.
10. Dog appeared better. Haemoglobinuria.
12. Haemoglobinuria continues.
18. „ „ Very few parasites.
22. No parasites found in the blood.
23. Dog appears well.
25. Dog killed. Autopsy: spleen large, kidneys inflamed, and many parasites found in kidney smears.

Result. Atoxyl treated dog (V) was killed on the 9th day, and was found to be suffering from a heavy infection. Dog VI died from piroplasmosis on the 16th day. The control dog had apparently almost recovered on the 25th day. (Although considered as a control by Gonder this dog received more atoxyl than any of the other animals.)

Gonder's experiments show that atoxyl does not favourably influence piroplasmosis in dogs. It does not retard the appearance of the parasites or delay the progress of the disease. In fact the symptoms appear to be aggravated. The drug by itself produces similar effects to piroplasmosis, haemoglobinuria, bloody stools, great inflammation of the kidneys, and death due to cardiac failure (see Uhlenhuth, Hübener and Worthe, 1908, p. 277), so that satisfactory results could scarcely be expected.

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NOTES ON NAGANA AND ON SOME HAEMATOZOA OBSERVED DURING MY TRAVELS.

By HERBERT E. DURHAM.

I. Nagana.

Several observations on the disease caused by the *Trypanosoma brucei*, which were made after the publication of the paper by Kanthack, myself and Blandford[1], have not yet been published, and since the points have not attracted the attention of other writers, it seems worth while to place them on record.

Longevity of guinea-pigs suffering from Nagana.

It was stated in the above cited paper that the longest period of survival, which till then had been observed in guinea-pigs, was 183 days. A still smaller maximum was observed by Laveran and Mesnil[2], namely only 61 days.

When the virus was first received from South Africa, the experiments with guinea-pigs at first led us to believe that this animal was refractory. The guinea-pigs which had been inoculated and brought to England survived and did not show any signs of infection. Similar failure occurred with the earlier inoculations, which were performed by us with blood from the successfully infected dog which was delivered to us[3]. Further experiment however showed that the guinea-pig is not refractory, and many observers have had no difficulty in maintaining the strain by means of this animal.

The following observations are of interest, as showing that guinea-pigs may possess a considerable degree of resistance to the trypanosome:—

Two guinea-pigs ("A" and "B") were inoculated with blood of

[1] *Proc. Roy. Soc.*, vol. xliv. 1898, p. 100, and *Hyg. Rundschau*, vol. viii. p. 1185.

[2] *Trypanosomes et Trypanosomiases*, Paris 1904.

[3] The syringe was used for all our earlier inoculations, later we used a surgical needle merely wetted with infective blood.

an infected horse on 3. v. 1897. Thereafter, though their blood was examined frequently, and though their temperatures showed occasional irregular rises, no trypanosomes were found in their blood. It may be presumed either that the infecting blood was at fault, or that they were resistant individuals. The sequel is somewhat in favour of the latter view. It may be noted here, that on the 43rd day after this first inoculation, rats were injected with the blood of each guinea-pig with negative result.

Guinea-pig "A" was reinoculated by the late Dr Kanthack on 10. II. 98 with blood of a guinea-pig containing active trypanosomes. Its blood was examined at intervals, and from 6. III. to 8. VI. 98 the presence of the trypanosome was noted. From that day onward trypanosomes were absent, so that on 24. XI. 98, this guinea-pig was again inoculated, the blood of an infected rat being used. The period covered was already more than nine months since the inoculation which had shown itself to be successful. Seventeen days after this third inoculation the blood was free from parasites, but by 24. II. 99 the trypanosome had become common, and the animal seemed to be ill. Eventually it died on 3. III. 99 or 99 days after the last inoculation. At the autopsy, the lymphatic glands were found to be exceptionally enlarged for a guinea-pig, the spleen was also much more enlarged than usual; in fact the appearances recalled those which obtain in the rat.

The further notes on guinea-pig "B" have unfortunately been lost.

Observations on another pair of guinea-pigs ("C" and "D") may be cited; these were first inoculated by Dr Kanthack on 22. II. 98 from an infected guinea-pig.

Guinea-pig "C" showed that it had been successfully infected, as trypanosomes were present in its blood 62 days later; eventually it died of the infection on the 102nd day.

Guinea-pig "D" is of greater interest. Trypanosomata were discovered in its blood 44 days after inoculation and again on 15. IV. and 25. IV. 98. Subsequent examination twice or thrice a month failed to reveal parasites. On 24. XI. 98 it was again inoculated, this time from a rat. Unfortunately on 20. II. 99 it was given another inoculation. On 8. III. 99 and again on 21. III. its blood was crowded with trypanosomes. Death occurred three days later. Counting from the original infection, the animal survived no less than 13 months, whilst the survival after the first reinoculation was 120 days; it may be noted that this infection was done from the same rat that served in the case of "A." It would seem then that although individual guinea-pigs may show

a considerable resistance to infection with the nagana trypanosome, yet they are not solidly immunised by apparent recovery from one attack.

Relation of Birds to Nagana.

In our joint report the fact is recorded that we were unable to infect pigeons with nagana; this has been confirmed by other writers, for instance Laveran and Mesnil.

It seemed possible that other kinds of birds might have a different reaction, and having obtained a kestrel (*Falco tinnunculus*), I inoculated it in the breast with citrated rat's blood, which swarmed with Nagana trypanosomes. The injection, about 2 c.c. in bulk, was given on 10. XI. 98. Twenty days later (1. XII.) the breast was punctured, and two rats were inoculated with the blood that was obtained, although no recognisable trypanosomes were to be seen. One of these rats died on the 12th day with trypanosomes in its blood and great enlargement of the spleen; its death, however, was complicated with a septic inflammation of the pleura. The second rat died on the 13th day, apparently of uncomplicated nagana, its blood was teeming with the parasites and the chief enlargement of the lymphatic glands corresponded with the site of injection (right thigh).

On 11. XII. the blood of the kestrel was again examined both locally from the breast and from the foot, but nothing that could be recognised as trypanosomal was found. Two more rats were injected with blood from the breast on this day, 31 days after the original inoculation of the bird. Both these rats succumbed to nagana; one was killed on the 11th day with teeming masses of trypanosomes in its blood; the other died on the 16th day. In both the chief glandular enlargement was in the right inguinal region, corresponding to the inoculation in the thigh.

On 24. II. 99 two more rats were inoculated, but they died after 24 and 48 hours of septic mischief.

On 6. III. 99 two more rats were injected with blood from the bird's breast. One of these died 35 days later without signs of infection by nagana, the other survived.

A reinoculation of the bird was performed on 25. IV. subcutaneously in the breast with about 1 c.c. of highly infected citrated rat's blood. On the next day only bird's and rat's red blood corpuscles, haemoglobin crystals and leucocytes could be found in a sample removed for inspection. Fifteen days later a rat was inoculated with blood from the breast, the result was negative.

On 16. VI. 99 nearly the whole of the blood (about 5 c.c.) of a highly infected rat was injected subcutaneously as before into the breast of the kestrel. On the next day a few active trypanosomes together with clumped masses of dark crinkled rat red corpuscles, leucocytes and granular matter were seen in a sample removed. A rat inoculated with this sample died in ten days of nagana. The bird was again examined locally three days after the injection, no trace of haematozoa or of rat blood corpuscles could be found; leucocytes however were abundant. Two rats inoculated died in 12 and 17 days of typical nagana.

On 30. VI. a rat was inoculated with negative results.

The kestrel remained in apparently good health. Owing to its fractious nature and the danger of injuring it, its temperature was not taken regularly, one day it was noted to be 41·7° C.; this is in accordance with the well known high temperature of birds.

In order to control this experiment with the kestrel, a *pigeon* was treated in exactly the same manner and contemporaneously. The dose given amounted to nearly 10 c.c. of citrated rat's blood (there were 2,500,000 trypanosomes per cubic millimetre in the undiluted blood).

Rats were inoculated on each of the four following days; only one of these died, death being within 48 hours apparently due to sepsis.

On 10. XI. 98 or 59 days after the first injection, a second inoculation of highly infective rat's blood was given. Rats were inoculated at various periods, all with negative result. The temperature record of the pigeon was rather higher than that of the kestrel, viz. 42·5° C.

Until actual observations have been made on birds of prey in nagana infested regions, it is only possible to say that they might conceivably be carriers of infection. Koch's observation that *Glossina* will feed on crocodiles is of interest in this connexion.

Remarks on the Pathogenic Action of Trypanosoma brucei

If the course of the disease as it occurs in rats is compared with that which obtains in the rabbit, it is clear that the trypanosomal form of the parasite, as such in the circulating blood, has but a small share in the determination of illness. Thus a rat may have several hundred thousand trypanosomes in each cubic millimetre of its circulating blood, without showing grave signs of its rapidly approaching death. The same is true of guinea-pigs, for I have watched a buck which was most zealous in its attentions to a doe, a few minutes after counting 500,000 trypanosomes per cubic millimetre in its blood; moreover the animal's

weight record showed a continuous steady gain in weight since the inoculation.

On the other hand, progressive wasting and local swellings make a rabbit into a truly melancholy sight long before death supervenes, yet even with the aid of the centrifuge, the presence of trypanosomes may not be discoverable, until within a few days of death. The following example may be cited: a rabbit (2650 grams) was infected with a needle wetted with infective blood, 28 days later the weight had fallen to 1440 grams, the nose and eyelids were much swollen and obstructed. It was killed and its tissues searched for the presence of trypanosomes. The blood revealed none, in fact the only place where the parasites were found was in the rib marrow, and there they were quite sparse. Bone marrow in other regions was examined with negative result. It might be thought that the presence of the parasite in so important a tissue as the rib marrow might be significant to explain the disease, were it not that the trypanosome occurs in great numbers in the similar marrow of the rat. It is, perhaps, not inapt to remark that in malaria, the mature or almost mature parasites do not assert their presence with the marked disturbance of health, which is caused by the shower of a young brood through the system.

In animals, especially when they are near unto death from nagana disease, the blood shows marked morphological changes; the red blood corpuscles are reduced to about half their proper number, the leucocytes may be considerably increased, and nucleated red corpuscles are to be found. In my experience nucleated red corpuscles appear more abundantly in the rat and mouse than in other animals. Thus on one occasion the blood count of a rat, which was moribund, gave the high figure, 4,500 nucleated red corpuscles and 28,500 leucocytes per cubic millimetre. At this late stage of the disease the red corpuscles do not run well into rouleaux but tend to aggregate into small clumps. This clumping of the red corpuscles is evidently due to an alteration in the plasma or serum, for healthy corpuscles are caused to aggregate in a similar manner by mixing them with the diseased serum.

In some respects, nagana recalls the condition known as pernicious anaemia in man; and it may be noted that in 1897, I inoculated several rats with blood taken from patients suffering from this complaint. Cases are rare and I was not successful in trying from a case, which had not had arsenic administered therapeutically. At the same time several rats were injected with blood from patients suffering

from various kinds of leuchaemia. None of these animals showed any deviation from health or signs of parasites.

There is another blood change in nagana which on the whole is best seen in the rat when in an advanced stage of the disease. This change is manifested by a change of colour of the blood from the brilliant scarlet to a dull purplish or chocolate colour. The contrast between the healthy blood and the diseased blood is very striking when it is kept from coagulating by means of citrate, and can be recognised at a distance of some yards. In the clotted condition the phenomenon is likewise apparent, though hardly so marked. This dull coloured blood may be shaken with air, or allowed to stand for a week or more without developing the full red of normal oxyhaemoglobin. By the addition of the diseased blood to healthy blood the colour of the mixture is more or less dulled, so that the presence of healthy haemoglobin is not capable of causing a discharge of the abnormal colour. Examination with the spectroscope showed the bands of oxyhaemoglobin. Dr F. G. Hopkins kindly examined a specimen and considered that the oxyhaemoglobin bands did not appear quite normal. Dr Haldane has kindly drawn my attention to some observations[1] on the blood colour in poisoning with nitrobenzol derivatives. It appears that some species exhibit such changes whilst others do not do so; thus nitrobenzol causes no blood colour change in mice, though it does in dogs and cats. With dinitrobenzol the blood of the rabbit becomes chocolate coloured; this seems in part due to the presence of methaemoglobin but some other abnormal pigment is also probably at work.

Considerations such as these lead to the thought that though we now know much of the life-history and morphology of the parasites, this knowledge has taught us little, if indeed anything, concerning the disease itself. The same may be said of malaria in which morphology has not availed to advance our knowledge beyond diagnostic and preventive measures, the real nature of the disease and its symptoms yet remain to be unfolded. Another blood change, which may be mentioned as a matter requiring further elucidation, was noted in nagana-cachectic rabbits and consisted in the comparative resistance to haemolysis by the quillaia saponins. These saponins (sapotoxin and quillaiic acid) are capable of haemolysing red blood corpuscles in high degree, the extent varying in different species of animals. If 1 % dilutions of normal blood are treated with the glucosides it is found

[1] Haldane, Makgill and Mavrogordato, *Journ. of Physiology*, xxi. Nitrobenzol and dinitrobenzol, p. 184.

that small additions of serum of normal blood have some sheltering or delaying action. Normal cat or dog serum has a greater delaying effect than normal rabbit serum, but it was found that the serum of rabbits in the highly cachectic condition of the later stages of nagana had a still greater effect. von Dungern attributes variation in this response to haemolysis to the amount of lecithin substances in the case of normal animals; such may also be the cause in nagana.

Summary.

1. Some cases of remarkable resistance to nagana infection are recorded.

2. Although such birds as pigeons are unable to harbour the trypanosoma brucei, the kestrel is able to do so.

3. Attention is drawn to certain changes which are brought about by the trypanosome infection and the need for more precise chemical investigation of these haematozoal diseases.

Notes on blood parasites observed in Christmas Island (Straits Settlements) and in the Malay Peninsula.

Christmas Island possesses quite a number of peculiar species in its fauna, and it is regrettable that observations were not made before animals had been imported to this isolated station, as well as that my own notes are so incomplete.

Rats. There are three species of rat (1) the large *Mus nativitatis* had already become rare about the settlement at the time of my visits and I was unable to obtain either a freshly killed or a living specimen although a reward was offered.

(2) *Mus macleari.* A number of these rats was obtained and examined, of these nine were collected about the settlement; all were males and two of them had abundant trypanosomes in their blood. The trypanosome appeared morphologically like the *T. lewisi* of English rats.

Two other *M. macleari* were caught on the top of Phosphate Hill, some three or four miles from the settlement, one was a male the other a female and both were free from infection. Another was taken about half way up the hill and showed abundant trypanosomes in its blood;

the coat of this specimen was very infested with fleas[1], but there were none of the small ticks which were found on other specimens.

It is, perhaps, noteworthy that the spleen of this rat was very markedly enlarged, as also were the superficial lymphatic glands. This animal was a rover and possibly had acquired its infection from imported *Mus rattus*. The presence of more lesion than occurs usually with *T. lewisi* infections led to a working hypothesis that the annihilation of native rats by imported ship rats may be due to the introduction of trypanosomes, which, finding a "virgin soil" to work upon, cause fatal epidemics. Unfortunately all the later specimens that were examined proved to be free of infection.

Out of 12 specimens examined 3 (or 25 %) were affected with trypanosomiasis.

(3) *Mus rattus*. The specimens were identified at the British Museum, they varied much in colour from the so-called "grey" to black. The manager of the Phosphate Company, Captain Vincent, informed me that these rats were first introduced to the Island in December 1899 by the SS. *Hindustan* in a cargo of hay; they had multiplied to very great numbers at the time of my visit 1901–1902, but apparently they remained about the settlement. Altogether 13 of these rats were examined haematologically, and six of them were found to be harbouring a trypanosome of similar appearance to *T. lewisi*. In regard to this parasite, the presumption is, that it was introduced.

A species of biting fly, much like our *Stomoxys calcitrans*, was also very prevalent about the settlement and might spread infection.

Bats. Two specimens of the large *Pteropus natalis* were examined and both had infection with a small malaria-like parasite in their red corpuscles. Sporulation was not taking place at the time of examination. The coats of the bats were full of a louse-like parasite. Other means of spread of the infection is to be found in the mosquitos. I took three species on the Island, *Culex alis* (nov. sp. Theobald) being a new one, *C. fatigans* (only few specimens seen) and *Stegomyia scutellaris* which was very common and active during the day; the activity of the latter during the sleeping period of the bats would favour their attack.

Birds. The small ground pigeon, *Chalcophaps natalis*, was examined

[1] *Loemopsylla nesiotes* sp. nov. (Jordan and Rothschild, *Parasitology*, vol. I. p. 1. 1908). The Hon. N. C. Rothschild has kindly informed me that all the specimens I obtained from *M. macleari* were of this peculiar species, so that an interchange of fleas from *M. rattus* is not proven.

once and found to be severely infested with a halteridium-like parasite, the specimen was taken near the settlement. Here again there is doubt whether the parasite existed as an original inhabitant, for a number of carrier pigeons had been introduced.

Carpophaga whartoni was not examined, and *Zosterops natalis* was examined but showed no blood parasite.

Of the three blood parasites, in rat, bat and pigeon, those of the rat and pigeon have probably been introduced, whilst that of the bat seems likely to have been an old standing native occurrence.

Whilst on the topic of animal infections it may be mentioned that I made a number of blood examinations on birds at Kuala Lumpur Federated Malay States, often with the kind help of Dr J. D. Gimlette. The ordinary pigeons showed heavy infection with *Halteridium*, so also did two pet specimens of a small green parrot. The sparrows and a small species of pie were all free of infection, though several of each were examined.

The observations on nagana were carried on with the aid of the Tsetse fly Committee of the Royal Society, and the other notes were made during the Beriberi expedition of the London School of Tropical Medicine.

NOTE ON THE POLYMORPHISM OF *TRYPANOSOMA GAMBIENSE.*

By E. A. MINCHIN, M.A.

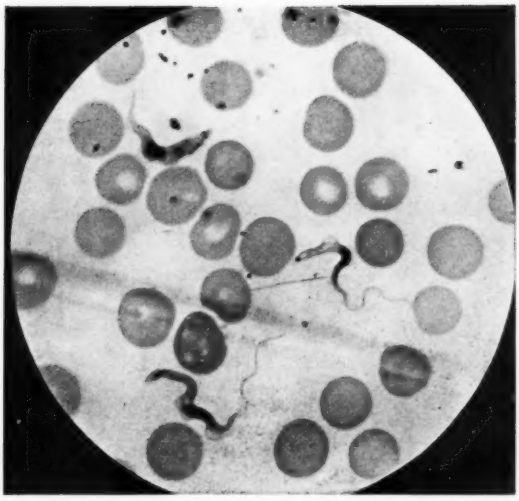
Plate XVII.

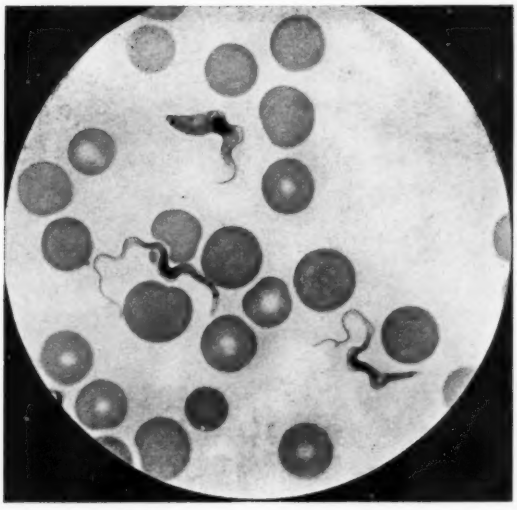
In a memoir recently published, Salvin-Moore and Breinl[1] state that when *Trypanosoma gambiense* is examined in the blood "it does not seem possible to detect any true dimorphism or trimorphism." "The three forms often described and alluded to as distinct, consequently appear to be arbitrarily chosen examples in a continuous series of dimensions." It has always seemed to me very remarkable that the great differences in form and structure, no less than in size, between the slender, ordinary, and stumpy forms of *T. gambiense*, differences noted by all competent observers, should have been denied by two authors who claim for their methods of technique a superiority over those employed by all other investigators.

In a preparation of *T. gambiense* from the blood of a rat, which was made by my friend and assistant Dr J. D. Thomson for the cabinet of the Protozoological Laboratory of the Lister Institute, the three typical forms of the trypanosome were found to be very distinctly differentiated. Dr Thomson found and marked two fields in which the three forms occurred in close proximity, so that it was possible to photograph them. The blood-smear was fixed wet with osmic vapour, stained with Giemsa's stain, and mounted in Canada balsam. The two photographs which are reproduced here were taken at a magnification of 1000 diameters by my friend Dr D. J. Reid.

It can be seen clearly from the photographs that the difference between the three forms of *T. gambiense* is by no means one merely of size. The slender form is of great length and has a very long free flagellum. On the other hand the stumpy form is short and its

[1] *Annals of Tropical Medicine and Parasitology*, I. (1907), pp. 450, 451; compare also II. (1908), p. 212.





flagellum is also very short, especially the free portion. The ordinary form, which is much the commonest on the slide, is more or less intermediate between the two extreme forms. There is nothing new in these statements, and it seems almost necessary to apologize for restating such obvious facts; it is to be hoped that it will not be necessary to do so again.

As regards the significance of these forms, I incline to the opinion that the two extremes, slender and stumpy, represent sexual forms differentiated in opposite directions from a neutral type. This view receives support from the observation made by me, that after about 12 hours in the tsetse-fly only slender and stout forms are to be found. Nevertheless the question of the meaning of this well-marked trimorphism cannot be regarded as definitely settled in the present state of our knowledge.

ON THE STRUCTURE OF "HALLER'S ORGAN" IN THE IXODOIDEA.

By Prof. G. H. F. NUTTALL, F.R.S., W. F. COOPER, B.A. and L. E. ROBINSON, A.R.C.Sc.

(Plate XVIII and one Text Figure.)

In the year 1881, G. Haller published a description of the sensory organ of ticks which has since borne his name; he was apparently so much impressed by its remarkable similarity in structure to the auditory organs of many Crustacea, that he assumed it to be an organ of hearing, and even went so far as to describe the presence of otoliths within one of the cavities of the organ. Since that time, other zoologists appear to have given little attention to this structure, the majority of those who have had occasion to mention it, contenting themselves with a brief resumé of Haller's observations and a reference to his paper.

The first to question the accuracy of Haller's interpretation of the function of the organ, was Lahille (1905), who published the results of numerous experiments made on living specimens of the South American Cattle-tick, *Boophilus annulatus* var. *microplus*. The conclusion which he drew from his observations was, that the function of Haller's organ is olfactory and not auditory. Lahille's observations concern only the function of the organ; he gives no anatomical description. Batelli (1891) calls attention to the fact that ticks frequently move their first pair of legs (bearing Haller's organ) in the manner of the antennae of insects, and suggests that the organs serve as a means of perceiving, at a distance, the presence of the host-animals which are so indispensable to their existence. He also points out the remarkable resemblance of Haller's organ to the sensory organs described by F. Dahl (1885), occurring in *Pachygnatha listeri*, but, after an examination of Dahl's description and figures, we think that the points of resemblance are somewhat remote. Other observers have alluded to the antenna-like movements of the first pair of legs in ticks (vide Wheler (1899, 1900), Hunter and Hooker

(1907), and Hooker (1908)). Regarding the structure of the organ, Lewis (1892) in a few cursory remarks on the subject, adds nothing to the information to be obtained from Haller's account. Martin (1895, p. 273, see Plate XIV, fig. 8) observed three vesicles on leg 1, in *Amblyomma quantini*: the median vesicle was described as spherical in shape and contained a small compact mass—apparently an otolith; the other two vesicles contained hairs only. Dönitz (1907) summarises, in three paragraphs, our knowledge of the structure and function of Haller's organ, up to that time.

In the course of our work on the Ixodoidea, we have investigated the anatomical structure of the organ in every genus, and we have come to the conclusion that Haller's description is erroneous in many respects, hence the present communication.

Throughout the entire super-family, the principal features of the structure of Haller's organ are sufficiently constant, that, for present purposes, a description of the organ as exhibited in *Haemaphysalis punctata*, after making due allowance for generic and specific differences in details, will suffice as a guide to all.

Haller's organ is a minute cavity or vesicle (diameter about 65 μ) containing sensory hairs and associated with specially modified hypodermal tissues which lie immediately beneath it. It is borne on the dorsal surface of the terminal article (tarsus) of the first pair of legs; Haller's statement that it is borne "auf der Bauchfläche der Extremität" is incorrect. It bulges down into the interior of the leg, its surface being flush with the surrounding parts, and is formed entirely of chitin which is continuous with the chitinous cuticle of the leg. The superficial part or roof of the vesicle is formed of thin transparent chitin, through which a minute slit-like pore opens (Fig. 1, p. 240), establishing communication between the interior of the vesicle and the external air[1]. In lateral view (Plate XVIII), as usually seen in mounted specimens of the tarsus,

[1] Knowing well the difficulty of correctly interpreting the real nature of minute structures formed of highly refractive chitin, especially in the case of a minute and thin-lipped pore, we have succeeded in demonstrating the patency of this opening in a fairly convincing manner. A leg of the first pair was snipped off a living tick, with scissors, and immersed in a syrupy solution of Canada balsam on a cover-glass: this was inverted over a small glass chamber mounted on a glass slip, in the manner of a hanging-drop preparation. A small lateral tube opening into the chamber made it possible to connect the apparatus with an air-pump, the excess of balsam on the cover-glass forming an efficient luting to form an air-tight fitting. The apparatus was placed on the stage of the microscope, and after sharply focussing the pore of Haller's organ, the air was slowly exhausted. In every case, immediately exhaustion commenced, a bubble of air was seen to exude from the pore.

the cavity of the vesicle is seen to be divided by folds or thickenings of its chitinous lining, into two chambers—a smaller superficial chamber from which the above-mentioned pore opens to the exterior, and a larger chamber, the floor of which, on the side towards the proximal end of the tarsus, is raised into a number of shallow conical papillae (Plate XVIII, Fig. 1), each of which bears a sensory hair. The sensory hairs are straight and stiff and protrude across the cavity of the larger chamber, their points being directed towards the irregular and indefinite opening between the two chambers. The sensory hairs are 20—25μ in length

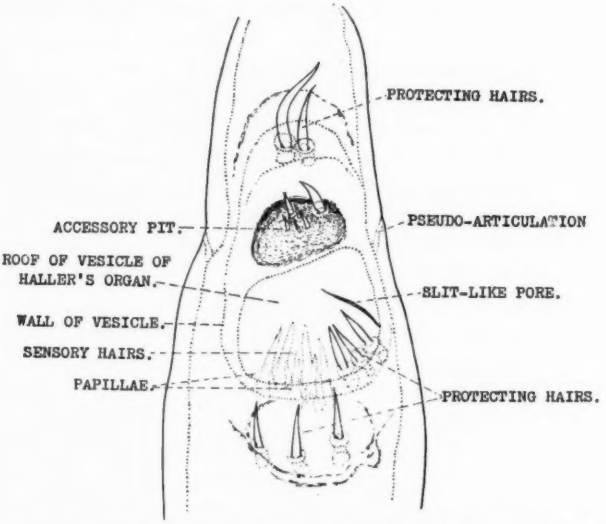


Fig. 1. Haller's organ as seen from the dorsal aspect. ×330. (L. E. R.)

and differ in their appearance from the other hairs on the body and appendages of the tick: the internal cavity of each is comparatively large, the bounding walls are unusually thin, and there is no constriction at the base, the contour of the shaft of the hair merging into that of the basal papilla without any perceptible line of demarcation.

With regard to the structure of the hypodermis underlying Haller's organ, we have been limited to a study of sections, the material for which was preserved by methods unsuitable for the determination of the structure of delicate cellular elements, and until new supplies of living

material are forthcoming, we are unable to complete our work on the histology of the soft parts. From the few observations which it has been possible to make, it appears that the hypodermal cells immediately beneath the sensory hairs are large and flask-shaped; each consists of a sac of finely granular cytoplasm surrounding a large central cavity or vacuole which communicates directly with the hollow interior of the hair. We have succeeded in identifying nerve fibrils running towards the bases of the sensory hairs, but cannot, at present, define their relationships with the modified hypodermal cells.

Closely associated with the vesicle of Haller's organ and included in Haller's description as an intrinsic part, is a pit-like depression which we have termed the *accessory pit* (Plate XVIII, Fig. 1): it lies a little distance beyond the distal side of the main vesicle, is widely open to the exterior, and is furnished on its floor with a number of short stiff hairs, some of which protrude beyond the mouth of the pit. This structure appears to have been considered as a small counterpart of the chief vesicle, but its appearance, together with the fact that the hairs which it contains are quite different in character from those of the latter, does not support this view. The surface of the leg on both the proximal and distal sides of Haller's organ bears a cluster of stiff hairs, the function of which is, presumably, protective (Text fig. 1).

As already stated, Haller alluded to the presence of an otolith in the cavity of the organ and a representation of such a body is clearly shown in his figure: we have diligently searched for such bodies in all our preparations but have failed to detect their presence and have no hesitation in denying their existence.

In conclusion, we are compelled to doubt that the function of Haller's organ is auditory. Haller based his interpretation on the presence of a supposed tympanic membrane, of otoliths, and the resemblance to the auditory organs of certain Crustacea. No one, as far as we know, has attempted any experimental proof of an auditory function. On the other hand, the structure of the organ, the peculiar antenna-like movements of the first pair of legs, and the results of Lahille's experiments are all strongly in favour of the assumption that Haller's organ is olfactory in function.

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DESCRIPTION OF PLATE XVIII.

Fig. 1. *Haemaphysalis punctata.* Lateral view of left tarsus of female. ×440. (L.E.R.) The figure is slightly schematised and represents the vesicle of Haller's organ and the accessory pit in optical section.

Fig. 2. *Haemaphysalis punctata.* ♀. Photomicrograph of Haller's organ from the side, showing the pore, accessory pit and part of the chitinous fold which differentiates the smaller chamber from the larger. ×150.

Fig. 3. As in Fig. 2. This photograph gives some slight indication of the slit-like character of the pore. ×400.

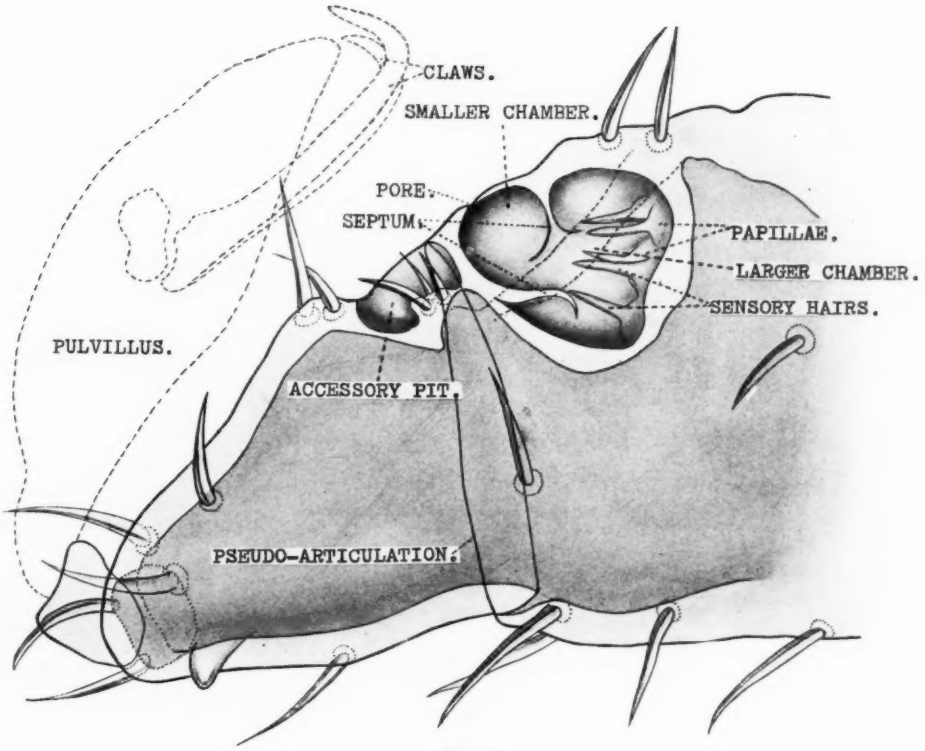

Fig. 1.

Fig. 2.

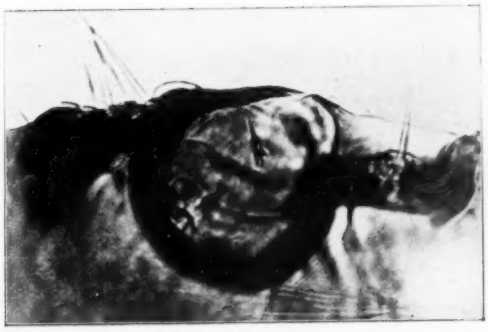

Fig. 3.

THE DEVELOPMENT OF PIROPLASMA CANIS IN CULTURE.

By GEORGE H. F. NUTTALL and G. S. GRAHAM-SMITH.

Plate XIX and 1 Text Figure.

In the course of their investigations upon different species of *Piroplasma* numerous observers have kept infected blood in the ice-chest, or at room temperature, for various periods of time prior to the inoculation of animals. On the evidence afforded by microscopic examination alone some observers have concluded that the parasites are capable of considerable multiplication in defibrinated blood kept *in vitro*. Under these conditions many corpuscles undergo haemolysis and in consequence the parasites appear to be more numerous. We believe that this source of error explains the conclusions arrived at by Lignières and others with regard to the multiplication of *P. bovis* in extravascular blood (see p. 253).

It is only within the last few years that systematic attempts have been made to *cultivate* the different species of *Piroplasma*. We have previously referred (1905, *Journ. of Hygiene*, v. 245) to the negative results which followed the attempts of Nocard and Motas (1902, pp. 274—275) to cultivate *P. canis*, although they established the fact that the parasites remained alive and virulent in blood which had been preserved in the dark and cold for 25 days. Kinoshita (1907, p. 111) has kept the parasite alive for 31 days on ice. In an earlier paper we have described the forms of parasites observed in defibrinated dog's blood up to 48 hours after its removal from the animal.

Kleine's observations.

Kleine (1906, pp. 10—15) states that most of his attempts to cultivate *P. canis in vitro* proved negative until he adopted Robert Koch's suggestion and studied the early changes which take place in cultures made by diluting defibrinated piroplasma blood with salt solution. Kleine infected young dogs by the intraperitoneal injection of about

10 c.c of defibrinated piroplasma blood. When the parasites became numerous in the blood of the infected dogs (they usually died on the third or fourth day) he bled the animals to death under chloroform, defibrinated their blood and added 0·5 c.c. of blood to 0·5 c.c. of salt solution. A series of 20 such cultures in test tubes were made at one time and kept at 27° C.

After the lapse of 18 hours Kleine decanted the clear supernatant fluid from the tubes and upon examining the deposit found that it contained bodies corresponding in form to those described by Koch (1905, 1906) in piroplasma infected ticks. Many of the parasites occurred in the form of clubs with about six rays protruding from the broader portion, whilst the tapering extremity also bore two or more radial processes. The parasites appeared peculiarly rigid, but, on closer examination in drop culture, amoeboid movements could be detected, the parasites becoming slowly rounded or elongated, whilst the length and number of the radial processes changed. These changes were already observable after eight hours, but were most marked after 18 hours.

After 48 hours some parasites had attained a length of 14 μ and a width of 4 μ, the rays at the broad end measuring 9 μ, those at the opposite end measuring up to 19 μ in length. These large parasites occurred together with parasites of normal size. When stained by Giemsa's method the large forms with radii showed a large chromatin mass at the end of the club, and usually a secondary chromatin mass at the tapering extremity. Kleine states that the rays appear blue, unless intensely stained when they take on a red colour. When the parasites were numerous in the blood, masses of radiate bodies occurred, the tapering extremities of the parasites converging to a common centre. When the parasites were very numerous the development of these large forms appeared to be inhibited. At times Kleine observed "fused forms" such as Koch has described in ticks, sausage shaped bodies with radii protruding from the extremities and containing large masses of chromatin at the ends of the sausage and secondary masses of chromatin situated about midway along their length.

After two days at 27° C. the parasites appeared on the whole larger than normal. In forms from early cultures both chromatin masses stain in the same way, but in those derived from cultures two days old the chromatin mass within the clubs retains the peculiar black-red appearance, whilst the larger chromatin mass surrounded by radii appears more lightly stained. After 2—3 days the parasites cease to

exhibit radii, they become rounded and an achromatic zone appears round the dark chromatin mass. The number of degenerating forms increases after the third day. The process of multiplication in defibrinated and diluted blood could not be observed, and no multiplication took place when the developmental forms were transferred to fresh normal or diluted blood.

Kleine thinks that in canine piroplasmosis the parasite usually lies upon the red blood corpuscle. We have elsewhere stated our reasons (*Journ. Hygiene*, VI. 636; VII. 250) for regarding these parasites as being intracorpuscular, and see no reason for changing our opinion[1]. Kleine also considers that the cultivation forms with radii are usually epicorpuscular, but here again we hold a contrary opinion. Owing to its extreme delicacy and lack of staining power the corpuscular envelope may perhaps have escaped his notice in many instances. Occasionally the corpuscular membrane cannot be demonstrated and then the parasite appears to be free.

The writers' experiments.

In our attempts to cultivate *P. canis* under artificial conditions we have made use of several different methods and media, which are described in the following pages. Under most of these conditions the parasites rapidly degenerated without exhibiting any signs of multiplication, or changes of form suggesting further development. These negative experiments (A—L) are first described.

In the last series of experiments (M) however certain large forms with long radiating processes were encountered, similar to those described by Kleine and Koch.

A. In order to ascertain whether the parasites would enter and multiply in normal dogs' corpuscles kept outside the body under artificial conditions the following experiments were made. Blood-stained *serum* taken shortly after death from the heart of a dog dead of piroplasmosis was centrifugalised, and drawn up into capillary pipettes.

[1] Kleine's statement and that of Kinoshita (1907, p. 309) regarding the epicorpuscular position of *Piroplasma canis* appears to be based upon deceptive appearances in stained preparations. Kleine's paper is illustrated by excellent coloured figures and microphotographs which show appearances essentially similar to those we have observed. We have not however seen large agglomerations of radiate bodies such as he figures on Plate IV, fig. 14, and Plate V, fig. 12.

Kinoshita apparently confined his attention to the study of stained preparations of *P. canis*. He has consequently advanced various hypotheses regarding the development of the parasite which we do not consider to be justified.

At the end of an hour the clear fluid contained some free motile pyriform bodies. Some of this fluid was now added to defibrinated normal dog's blood and unstained preparations of the mixture thus obtained were examined. Although free swimming parasites were kept under observation, with one exception which we have previously described (*Journ. of Hygiene*, 1906, Vol. VI. p. 632), none were seen to enter fresh corpuscles.

In other experiments some of this fluid was mixed with normal dog's corpuscles suspended in sodium citrate solution, and the mixture kept in the dark at room temperature for 20 hours. At the end of this time fresh preparations were examined and motile parasites seen, but none were found in corpuscles. Stained specimens confirmed the observations made on living preparations.

Similar experiments were carried out with serum obtained from blood which was allowed to clot, with the same results.

B. Dog's serum containing motile pyriform bodies was added in small quantities to tubes containing rabbits' blood corpuscles suspended in 4 % sodium citrate solution. Examinations of fresh and stained preparations showed that the parasites had not entered the corpuscles.

C. The following experiments were undertaken to determine whether the parasites would live and multiply in the serum of infected animals. Shortly after death blood-stained serum from the heart was drawn up into tubes and centrifugalised. Two hours later the clear fluid showed many rounded parasites and a few actively motile pyriform bodies. After 17 hours a few rounded forms were found, but no motile parasites.

Serum taken from coagulated blood was examined in the same way. This also showed some rounded forms and a few motile pyriform bodies after two hours, but no motile forms after 22 hours.

D. Several experiments were made with *defibrinated blood* taken from the heart immediately after death. Some samples were kept in sealed tubes, and others in tubes plugged with cotton wool. Some of these tubes were kept in the ice chest, and others at room temperature. Examinations of fresh and stained preparations were made at various times with samples taken from the various layers into which the fluid separated. In all 15 experiments were carried out and the examinations were made at times varying between 17 hours and 69 days. Motile pyriform bodies were noticed up to 17 hours, but not later, and non-motile pyriform bodies were seen up to the third day. After this time all the free parasites seen were either rounded or in various stages

of degeneration. The majority of the intracorpuscular forms soon became rounded, but a few of the pyriform ones retained their shape for many days. Occasionally large numbers of rounded parasites were found within leucocytes. (See Plate XIII, fig. 3, *Journ. of Hygiene*, Vol. VI. 1906.)

In some cases the red blood corpuscles retained their normal shape up to 35 days.

E. Eleven experiments were made with blood agar in the hope of obtaining cultivation forms. The blood agar was made by mixing equal parts of defibrinated rabbit's blood with melted agar at 55° C. and was prepared one hour before use. Small quantities of fresh defibrinated heart's blood of dogs dead of the disease were placed in the tubes, which were cultivated at various temperatures. Examinations made at various times revealed only a few degenerated parasites.

F. Defibrinated dog's blood added to various quantities of 2% sodium citrate solution was kept under the same conditions and examined in the same way as in experiment D. Seven experiments were made, the times of the examinations varying between 28 hours and 72 days. Even after 28 hours the majority of free forms and intracorpuscular parasites had become rounded. After this time very few free parasites were seen. In one experiment a few forms with well marked slender radiating processes like those described later (p. 252) were seen. In some cases the red blood corpuscles were found well preserved up to 53 days.

G. Five similar experiments were made by adding defibrinated blood to slightly acid 2% sodium citrate solution with the same results.

H. In three experiments defibrinated blood was added to 4% sodium citrate solution and the cultures examined at various times between 13 hours and 23 days. With very few exceptions both the intracorpuscular and free parasites were found to be rounded. Many of the red blood corpuscles retained their shape up to the 23rd day.

I. Two experiments with normal saline solution containing 4% sodium citrate gave similar results.

J. In six experiments defibrinated blood was added to 25% potassium oxalate solution and specimens were examined between 36 hours and 62 days. The same changes were noted as in experiment F. In some cases the red blood corpuscles retained their normal shapes up to 60 days.

K. Six experiments with slightly acid potassium oxalate solution gave the same results.

L. Following the method of Miyajima (1907) (see p. 255) numerous cultures were made by mixing defibrinated blood and ordinary nutrient broth in proportions varying between 1—5 and 1—10. These were kept for various periods at temperatures ranging between 18° C. and 35° C. Though the red blood corpuscles were well preserved the parasites rapidly became rounded and lost their motility. No developmental forms like those described by Miyajima were seen and none with radiating processes.

M. In the series of experiments about to be described certain very large irregular intracorpuscular forms with radiating spike-like processes were occasionally met with, apparently identical with the cultural forms described by Kleine, and resembling the free parasites observed by Koch in the early stage of infection in the tick.

In the following description these are spoken of as forms with radiating processes and the processes as radii.

In all the following experiments the cultures were made by adding a quantity (about 0·5 c.c.) of blood, defibrinated by shaking with glass beads for 20 minutes, to an equal quantity of 0·6% or 0·8% salt solution or to a physiological saline solution (hereinafter referred to as "P" solution) with the following composition:

Sodium chloride	0·95%
Potassium chloride	0·025%
Calcium chloride	0·02%
Sodium hyd. carbonate ...	0·15%
Dextrose	0·1%
Distilled water	100 c.c.

Dog I. The cultures were made in 0·6% salt solution. Some of these cultures were kept in test tubes plugged with cotton wool, and others in Petri dishes, slightly tilted, at various temperatures (14° C., 16° C., 20° C. and 32° C.). Examinations of fresh and stained preparations were made at various times. At 14° C. after 20 hours a few forms showed short blunt processes. At 16° C. no forms with radiating processes were seen. At 20° C. after 16 hours one form with short radii was found, but at 32° C. several parasites showing radii were observed after 24 hours. These experiments seemed to indicate that a temperature between 20° C. and 32° C. was the most suitable for the development of forms with radiating processes, and consequently in the subsequent experiments the cultures were kept at 24° C.—26° C.

Dog II. Cultures were made in 0·6% salt solution and were kept

at 26° C. After 20 hours a few forms such as are depicted in Plate XIX, figs. 2—4, were seen in corpuscles which had lost their haemoglobin, but none with true radii were found.

Dog III. Several cultures were prepared in 0·8% salt solution and examined at various times, but no forms of interest were met with.

Dog IV. Cultures were made in 0·6% and 0·8% salt solution and kept at 24° C. for 27 hours. Several preparations made from the surfaces of the corpuscular layers of various cultures all showed numerous parasites with radiating processes from which the specimens shown in Plate XIX were drawn.

Preparations made from the other layers and the supernatant fluid showed very few if any parasites with radii.

Dog V. Ten cultures were made in 0·6% salt solution and kept at 24° C. Examinations at 17 and 25 hours were all negative as regards parasites with radiating processes. After 41 hours a single form with true radii was found.

Dog VI. A very complete series of cultures and examinations were made in this case. Cultures in 0·6% salt solution were kept at 24° C. Preparations made after 15 hours and after 25 hours showed no parasites with radii. Examinations after 43 hours, however, showed numerous large forms and some with true radii. These forms were still present a few hours later.

No forms of interest were noted in cultures made with 0·8% salt solution after 25 hours. After 43 hours several large forms were seen and one with true radii. Five hours later these forms were more numerous.

In "P" solution no interesting forms were seen after 15 and 24 hours. After 43 hours three forms with radiating processes were noted, and a few after 48 hours.

Dog VII. In this case all cultures were kept at 24° C. and were left absolutely undisturbed until they were examined.

In 0·6% salt solution after 28 hours parasites were numerous and a few forms with radii were seen. After 27 hours' cultivation no obvious changes were noticed, but after 73 hours many forms with well marked radiating processes were seen.

In 0·8% salt solution a few forms with radii were seen after 28 hours, and after 47 hours they were numerous, but after 73 hours their numbers had decreased.

In "P" solution no forms with radii were seen after 28, 47 and 73 hours' cultivation.

Dog VIII. In this case all the cultures were kept at 24° C. In 0·6 % salt solution after 18 hours large forms were numerous, some of which had radiating processes.

Examinations made after 32 and 48 hours' cultivation showed many large forms, a few of which possessed well marked radii.

In 0·8 % salt solution and in "P" solution large forms were seen, but none with true radii.

Dog IX. Cultures were made in 0·8 % salt solution and were kept at 24° C. After 30 hours many large pyriform bodies were seen, a few of which showed radiating processes, but after 44 hours both the large forms and those with radii were less numerous. Similar cultures in 0·6 % salt solution and in "P" solution showed no forms with radiating processes.

Dogs X, XI, XII, and *XIII.* Similar experiments were made with blood derived from these animals, but no forms with radii were seen.

Dog XIV. Cultures made in 0·8 % salt solution and kept at 24° C. for 26 hours showed numerous forms with well marked radii. Motility was observed in some of them.

Dogs XV, XVI, XVII, XVIII, and *XIX.* Similar experiments were made with the blood of these animals but no forms with true radii were seen.

From the foregoing account it can be seen that in some experiments numerous forms with radiating processes were encountered, whilst in others, apparently conducted under exactly similar conditions, none were found. We have been entirely unable to find any cause for these differences in the behaviour of the cultures.

The differences apparently do not depend on the age of the animal from which the blood was taken, the height of the fever, or the rapidity with which it developed. Nor do they appear to depend on whether the temperature was falling or stationary, or on the period at which the blood was taken either in regard to the date of inoculation, or to the time at which the fever appeared. The following table summarises the data on which these statements are based.

The forms with radiating processes were found most commonly in cultures made in 0·6 % salt solution, and were most frequently obtained from the uppermost part of the layer of blood corpuscles. Although it appears to be necessary to use small quantities of fluid, probably in order to ensure the presence of a sufficient oxygen supply, we did not find that better results were obtained in shallow tilted dishes, with extremely shallow layers of fluid. Cultures which were left absolutely

at rest gave better results than those which were occasionally or continuously shaken, or through which a current of air was intermittently passed. The times at which the forms with radiating processes were most numerous varied in the successful cultures. In dog VIII they were numerous after 17 hours' cultivation, and were fewer after 44 hours. In most cases however they were most numerous on the second day. After that time they usually decreased in numbers.

Positive experiments.

Dog	Age	Temperature: At death	Temperature: Mode of rise	Condition when blood taken	Date after inoculation	Days after fever commenced
I.	puppy	103 °F.	—	stationary	3 days	2 days
IV.	,,	103·8	slow	falling	9 ,,	4 ,,
V.	,,	105·8	,,	stationary	7 ,,	5 ,,
VI.	,,	106	,,	,,	6 ,,	2 ,,
VII.	,,	104	,,	,,	4 ,,	0 ,,
VIII.	,,	103·8	,,	,,	6 ,,	2 ,,
IX.	,,	103·8	rapid	falling	9 ,,	1 ,,
XIV.	,,	103	slow	,,	6 ,,	2 ,,

Negative experiments.

II.	puppy	101·4° F.	slow	falling	12 days	6 days
III.	,,	103	rapid	stationary	10 ,,	2 ,,
X.	,,	—	?	,,	5 ,,	1 ,,
XI.	2 years	104	rapid	,,	4 ,,	2 ,,
XII.	old	103·8	,,	,,	5 ,,	1 ,,
XIII.	puppy	103·6	,,	,,	5 ,,	1 ,,
XV.	,,	103·6	,,	,,	6 ,,	1 ,,
XVI.	,,	102·2	very little	,,	7 ,,	3 ,,
XVII.	,,	104	rapid	,,	4 ,,	1 ,,
XVIII.	,,	103·6	slow	,,	5 ,,	2 ,,
XIX.	,,	—	—	—	—	—

Description of the cultivation forms.

In cultures made in 0·6 % and 0·8 % salt solution forms of the parasite may often be seen, which apparently are not degeneration forms, and which are never met with in the circulating blood or in the organs. The majority of these are large intracorpuscular forms. In very many cases the infected corpuscle has lost its haemoglobin, and its contour can only be made out with difficulty by following the faint line marking the rim of the collapsed and often much enlarged corpuscle. Occasionally no corpuscular remains can be defined and the large parasite appears to be free. Nevertheless we are inclined to think that

most of these apparently free forms are really intracorpuscular, and that failure to find the corpuscular envelope is due to its extreme delicacy and lack of staining power.

The least differentiated forms are large definitely intracorpuscular parasites, such as are figured in Plate XIX, figs. 2—4. Some of these show two well defined masses of chromatin (fig. 1) while others, usually of irregular shape, show three or more masses (figs. 2, 3, 4). Very rarely extremely large forms are seen with several masses of chromatin apparently connected together by thin strands of chromatin (fig. 21). Other examples of intracorpuscular parasites of the same general type show minute, delicate, radiating processes (previously described as radii) projecting beyond the parasites, and usually originating in the neighbourhood of a chromatin mass (figs. 5, 6). Others show much longer processes, frequently long enough to reach the margin of the corpuscle (figs. 7, 8, 9). These processes, which are often extremely delicate, especially at their distal extremities, vary greatly in number, sometimes being almost too numerous to count (fig. 12).

Occasionally several large parasities of this type are seen in one corpuscle (fig. 20).

In yet more remarkable forms some of the processes cause small projections or even considerable distortions of the corpuscular envelope (figs. 9, 11, 13), and occasionally may even perforate the envelope and project for a considerable distance beyond it (figs. 13—16). Similar forms (figs, 17, 18, 19), apparently free, are occasionally seen.

In living preparations all intracorpuscular parasites, except some of the rounded forms, show slight movements, probably of a molecular nature, since small masses of detritus with active dancing movements are often seen within the envelopes of the collapsed corpuscles.

On several occasions we have kept under observation living examples of the forms with long processes and have noticed changes of shape accompanied by the very slow protrusion and retraction of the radiating processes. The accompanying figure illustrates the changes noticed in one parasite (A) during 50 minutes' observation, and in another (B) during a period of five minutes.

Owing to the uncertainty of the cultural methods and the difficulty of keeping living parasites under observation for long periods we have been unable to follow the development of these forms. Possibly the forms with numerous processes develop from forms with many chromatin masses (fig. 21) and the latter by fusion from multiple intracorpuscular forms such as are illustrated in fig. 20.

In the absence of observations on living parasites the interpretation of the appearances seen in stained preparations is extremely hazardous[1] and we do not feel justified in offering any conjectures as to the origin and significance of the bodies we have described.

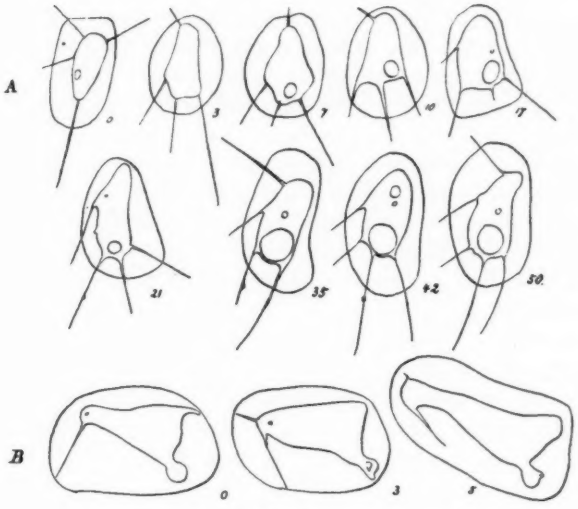

Fig. 1.

APPENDIX.

1. *Attempts to cultivate the other species of Piroplasma.*

Lignières (cited by Chauvelot, 1904, p. 11) using blood containing many parasites did not succeed in cultivating *P. bovis* in different kinds of broth, gelatin, agar, or on potato, serum, amniotic fluid, or aqueous humor, whether the cultures were exposed to the air, kept in an atmosphere of carbon dioxide, or hydrogen, or kept *in vacuo*. He also obtained negative results with blood kept in collodion sacs. As a culture medium he also tried serum rich in haemoglobin obtained from diseased cattle, adding to it 0·25 c.c. of blood containing many parasites,

[1] Breinl and Hindle (1908) have recently asserted that *P. canis* multiplies in the blood in several different ways. Observations on the forms assumed by the parasite in the living blood during multiplication, which can be studied without great difficulty, do not lend any support to their hypotheses, which are based on the evidence derived from wet films stained by Breinl's method.

which had shown "marked multiplication" at the bottom of tubes containing blood. After 15 days, according to Lignières, the parasites in haemoglobin serum showed marked multiplication and a third subculture gave even better results than did the second. All the culture forms were spherical and he states they contained one to four germs which leave the parent cell, grow rapidly and often remain in pairs connected by a filament which cannot be demonstrated by staining. Lignières (1903, Pl. IV) illustrates such forms in a later publication but his deductions appear to be so largely hypothetical that we do not deem it necessary to consider them further.

Dschunkowsky and Luhs (1904) tried to cultivate the parasites in haemoglobin serum in test tubes. A grayish deposit, which could be shaken up, formed at the bottom of the tubes. They thought they observed evidence of great multiplication at the end of 10—20 days. The parasites also appeared to multiply in defibrinated blood both inside and outside the red blood corpuscles. The parasites were definitely motile and appeared to multiply up to 20—25 days both at room temperature and at 38° C. (It was supposed at the time that they were working with *Theileria parva*.)

Piroplasma ovis.

Motas (1904, p. 31) attempted to cultivate this parasite in blood, blood serum and haemoglobin serum at different temperatures. His results were all negative. He notes that the blood gradually loses its virulence when preserved at 15—18° C., but may remain virulent up to 10—14 days. The blood remains virulent for longer periods, up to 15—20 days, if maintained at lower temperatures, provided no bacterial growth takes place. If the tubes become contaminated virulence is only retained up to 5—10 days. The parasites did not live nearly so long when citrate or oxalate of potash were added to the blood. The virulence was not affected by an exposure of 12 hours to minus 5—6° C. or by exposure to 43—44° C. for the same time.

Piroplasma equi.

Theiler (1903, p. 99) states that he has on several occasions kept the blood of horses containing this parasite *in vitro*. The blood was placed in test tubes and kept at different temperatures, in the ice box, at room temperature, and in the incubator. In the incubator the parasites disappeared from the blood as soon as the corpuscles lost their

haemoglobin. When the blood was kept at room temperature they persisted longer. In blood kept in the ice box the parasites after 12 days appeared as distinct as in fresh blood, but in most cases had become spherical, and were situated at the edges of the infected blood corpuscles. Very exceptionally free parasites were encountered. Some of the corpuscles which contained parasites did not stain in the vicinity of the parasite, so that the latter appeared surrounded by a colourless zone. Theiler suggested that this zone might be due to the destruction of the haemoglobin by some excretion product of the parasite. Although he transplanted the parasites into fresh serum he was unable to observe any multiplication such as Lignières states he observed in the case of *P. bovis*. He regarded the parasites which stained well after 12 days *in vitro* as alive.

Bowhill (1905, p. 2) mixed infected horse blood with potassium citrate in a flask and kept it at room temperature. After 24 hours many circular and oval extra-corpuscular parasites arranged in irregular masses were seen. Stained by the Romanowsky method the chromatin and protoplasm stained red and blue respectively. On adding fresh serum to infected blood and keeping the latter at room temperature, at 29° C. or at 40° C. he was unable to detect any multiplication of the parasites, although on one or two occasions he observed amoeboid movement.

Theileria parva (= *Piroplasma parvum*).

We have elsewhere (IX., 1908, p. 516) stated our reasons for excluding this parasite from the genus *Piroplasma*, but since it belongs to an allied genus it is desirable to consider the cultivation experiments of Miyajima (1907, p. 84).

This observer, working in Japan, states that he tried to cultivate the parasite in blood agar, sodium citrate (acid and alkaline), beef extract, peptone water, calf-serum, normal saline solution, and common broth, to which media he added infected blood. He obtained positive results when he added *Theileria* blood to ordinary broth in the proportion of 1—5 to 1—10 and maintained the cultures at 20—30° C.[1]

"The development of the parasites in a successful culture takes place in the following manner: on the first day no motile form is seen;

[1] Miyajima states incidentally that he was able to cultivate *Tr. lewisi* under similar conditions. Although we have tried to cultivate this parasite on several occasions according to Miyajima's method we have not succeeded in doing so.

on the second, there can be observed a certain number of peculiar cells which occupy the upper layer of the sedimented corpuscles and which microscopically appear as a series of white dots. Very few motile forms resembling trypanosomata are visible in these cells on the third day of incubation, but thereafter the trypanosomata multiply vigorously and reach the maximum number between the tenth and fourteenth day."

"In a culture kept at room temperature, the trypanosomata remain motile until 45 days later, at this time most of them have undergone degeneration and globular cells with irregular granulation result."

When kept at 10—20° C. the trypanosomes were alive after a lapse of three months.

Miyajima states that he was able to maintain the parasites alive in subcultures as Novy and others had done with *Tr. lewisi.*

According to Miyajima *Theileria parva* therefore appears to develop into a trypanosome under cultural conditions in blood added to broth. Nine of the 21 native cattle examined showed *Theileria parva* and from the blood of seven out of the nine trypanosomes were obtained in cultures. The transplantation of a single loopful of blood was sufficient to secure a positive culture. The development described by Miyajima appears to be extremely rapid. After three days at 25° C. the diminutive *Theileria* is stated to have attained or exceeded the size of a red blood corpuscle, and to have become actively amoeboid. Very large vacuolated cells appear after 20 hours and curious crescentic bodies after 48 hours which give rise to typical flagellates. After 72 hours besides the nucleus and blepharoplast the flagellates exhibit an undulating membrane and can be seen to divide longitudinally, gradually giving rise to large colonies.

As controls Miyajima examined 200 cattle but *Theileria* were not found in blood films and trypanosomes did not develop in cultures.

Miyajima confirmed the observations which have been made in other countries that *Theileria* cannot be transmitted to clean animals by blood inoculations. On the other hand he found that two out of three clean calves inoculated with cultures containing the trypanosomes became infected in eight days with red-water. One of these animals gave a positive result with cultures (development of trypanosomes) 17 days before the appearance of *Theileria* in its blood upon microscopic examination. If Miyajima's observations are correct they are certainly most interesting and remarkable, but a certain amount of scepticism appears justified until they have been extended and confirmed by other workers. It will be remembered that Schaudinn (1904, p. 438, see

Journ. Hygiene, VI. p. 642) advanced the hypothesis that *P. bovis* undergoes development into a trypanosome, a statement which has gained no support from subsequent investigations. Should *Theileria* develop into a trypanosome we would have an additional ground for separating it, in the light of our present knowledge, from *Piroplasma*.

2. *Blood-platelets and haematozoa.*

In connection with these cultivation experiments Swingle's (1908) observations on the similarity between blood-platelets and certain haematozoa are of interest. He made cultures of normal sheep and other blood in a medium of the following composition: water 1000 c.c., sodium citrate 5 grms., sodium chloride 5 grms. made slightly acid with hydrochloric acid. One drop of fresh blood was added to 2 c.c. of the solution. For making stained preparations he placed a drop of the culture fluid on a slide and after it had evaporated down, but was not entirely dry, he dropped on some killing fluid such as Zenker's solution. The specimens were stained by various methods.

He remarks that "normal platelets resemble normal piroplasma forms in size and general shape. A nucleus, and refractive spots, probably vacuoles, can be seen" (p. 49). We cannot entirely agree with this statement, since in our own preparations we have seldom found any difficulty in distinguishing between blood-platelets and forms of the parasite.

Swingle's observations, which are quoted at length, on the cultivation forms of platelets are extremely interesting. "Cultures examined as quickly as possible after drawing the blood showed amoeboid and flagellate forms. A fact of great importance to the student of haematozoa is that the most of these forms are capable of movement. They seem to roll over, swing round, and often move for a distance equal to the diameter of two or three red corpuscles. Among the amoeboid types there were always present in great numbers platelets with few or several long, sharp, or sometimes blunt, pseudopodia. From the description and figures of Koch and Kleine one must conclude that these forms are very similar to, if not identical with, what they describe as developmental stages of *Piroplasma*. To be sure Koch found his stages in the stomach of the tick, but this does not disprove the statement, for just such forms were also found in the stomach of the sheep tick after sucking the blood of a sheep....In older cultures most of the platelets that had no pseudopodia were at the rim, either inside or just

outside of a transparent circle about the size or a little larger than the platelet. Those outside looked as if they had crawled out of a thin envelope. As to their significance, I can only say that they apparently were not degeneration forms, inasmuch as they were still active in their amoeboid movements. The more typically flagellate forms, those with a single flagellum, are perhaps of still more importance, because they so perfectly simulate real flagellates. Although they are found immediately after the introduction of the blood into sodium citrate solution, the 'flagellum' is generally quite rigid except at the very end, where it can be seen to vibrate. Notwithstanding this rigidity, they seem to move about, roll over, and swing round, these movements probably being the result of the vibrations at the tip of the flagellum. The most motile forms were found in a culture of human blood kept for the first six hours in an ice chest and after that at room temperature for 50 hours. Round or pear-shaped individuals with a flagellum measuring in some cases as much as 20 μ were found in abundance. In the pear-shaped forms the flagellum is at the pointed end. It was very slender, in most cases appearing and moving very much like the flagellum of *Euglena*, often with lashings violent enough to move the red corpuscles on coming in contact with them. Instead of being smooth, in some instances the flagella had thickened, knotted portions, which bear a close resemblance to Kinoshita's description and drawings of the flagellates which he found. It is important to note that he found the best developed flagellate with a flagellum 15 μ long in sodium citrate culture.

The various forms are often found grouped together in couplets, triplets, or in masses composed of as many as a hundred individuals. In this condition they retain their individual motion, rolling over and turning about. I have seen these masses stained with iron haematoxylin so that they had the exact appearance of Kleine's photograph."

Swingle says that he found such masses in the stomach contents of sheep ticks fed on sheep's blood. "To eliminate the possibility of confusing the platelets with the herpetomonadine flagellates, which are generally present in adult ticks, they were studied in young ticks before the latter had become infected."

"Flagellation of blood-platelets is not limited to sodium citrate culture, but may take place in other media."

"In stained preparations one often finds a most striking, yet doubtless merely coincident, resemblance in nuclear conditions to trypanosome forms. While nuclear dimorphism is not by any means to be

found in all blood-platelets, yet it is by no means rare. And when it does occur there is not such marked distinction between the nuclear masses as one sees in trypanosomes, but still as much distinction as many of Kinoshita's drawings would indicate for *Babesia*....Thus it would be an easy matter to mistake such blood-platelets for real flagellates having true nuclear dimorphism." "The flagella and pseudopodia stain like cytoplasm, and not like chromatin as in the trypanosomes."

Swingle concludes his paper with the following words: "It is not sufficient answer to the similarity I have shown to say that Kleine used defibrinated blood, and hence blood-platelets were not present in his solutions, for no one has demonstrated that the platelets are entirely removed by defibrination. Since blood-platelets in various culture media and in the stomach of the tick always develop flagella, move about, and manifest such a marked resemblance in form, size, and structure to *Babesia* and the Leishman-Donovan bodies, investigators must furnish criteria to differentiate between the flagellated platelets and the parasites. Until they have established their position by experiments with normal blood, the correctness of their results can be accepted only with some reserve. The evidence I have presented shows that neither are motion and flagellation exclusive characters of parasites nor will they differentiate them from blood-platelets. Each student will have to determine experimentally how to distinguish the two classes of structures."

As controls for our observations we have carried out a number of experiments by Swingle's method, using *defibrinated* blood from normal and infected dogs. Occasionally we have found platelets such as he has described, but the majority of cultures showed none. We do not think that the intracorpuscular forms of piroplasma with radiating processes could be mistaken for platelets, and up to the present we have not met with any "flagellated" platelets which could be mistaken for the extracorpuscular parasites.

DESCRIPTION OF PLATE XIX.

Fig. 1. Large free pyriform parasite with two chromatin masses.
2, 3, 4. Large intracorpuscular parasites each with several chromatin masses.
5, 6. Large intracorpuscular parasites with short radii.
7. Small intracorpuscular parasite with several radii.
8, 9. Intracorpuscular parasites with long radii.
10–16. Intracorpuscular parasites with numerous radii, some of which apparently penetrate the corpuscular envelope.

Figs. 17–19. Large free parasites with long radii.

20. Photograph (×3000) of corpuscle containing several parasites with short radii.

21. Photograph (×3000) of corpuscle containing a large triangular parasite with several chromatin masses connected by thin strands.

22, 23. Photographs (×3000) of corpuscles containing large pyriform parasites with several long radii projecting from their blunt extremities.

24. Photograph (×3000) of corpuscle containing a large rounded parasite with numerous radii.

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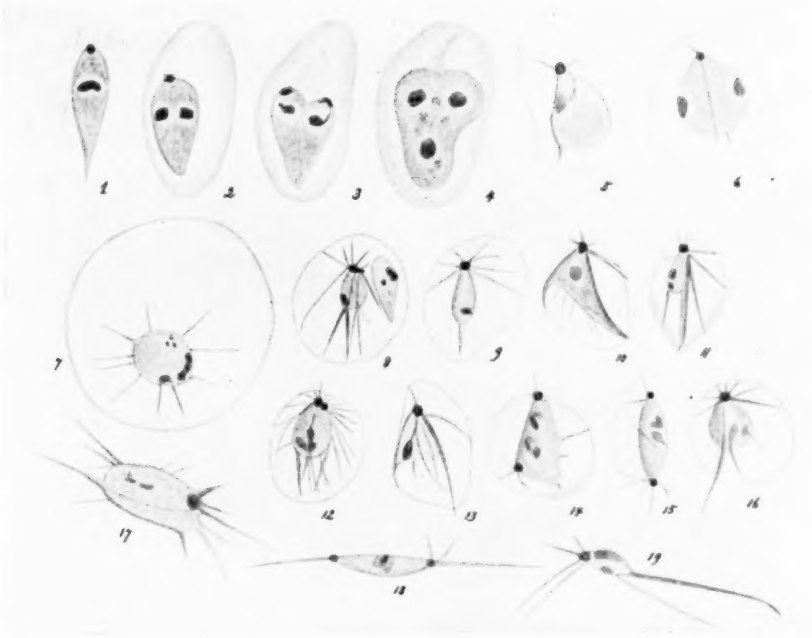

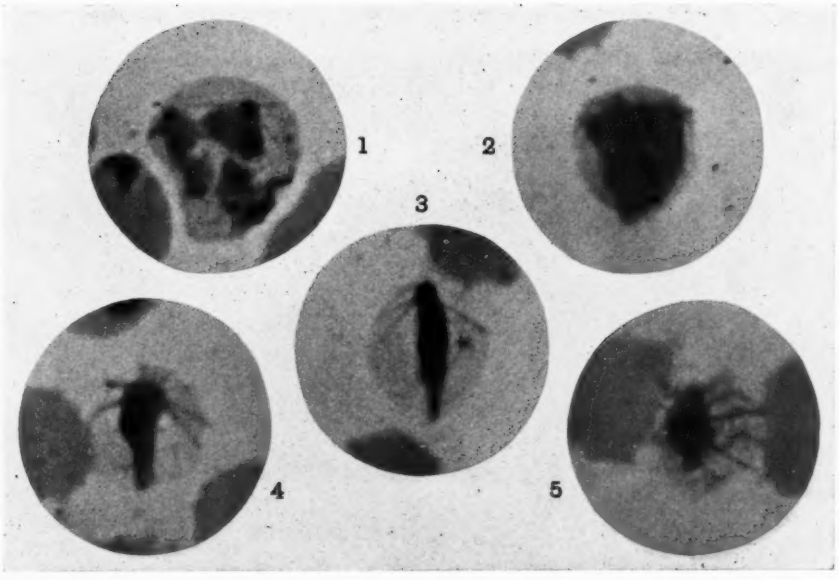
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NOTE ON THE PREVALENCE OF INTESTINAL WORMS IN DOGS IN CAMBRIDGE.

By GEORGE H. F. NUTTALL and CYRIL STRICKLAND.

In the course of investigations conducted in Cambridge upon dogs infected with *Piroplasma canis*, a number of animals have been incidentally examined for intestinal parasites. In an unbroken series of 24 dogs, which died (March—September 1907) from piroplasmosis, the animals were autopsied immediately after death and the intestine examined by slitting it open along its whole length. We briefly record the results of these examinations, for the reason that similar reports do not appear to have hitherto been published in this country. All of the dogs harboured worms. Only three species of worms were encountered: *Ascaris mystax*, *Dipylidium caninum* and *Taenia serrata*.

The parasites were distributed as follows:—

	No. of dogs harbouring each species
A. mystax	17
D. caninum	14
T. serrata	4

The species occurred either alone or associated with each other:

10 dogs harboured *A. mystax*.
5 „ „ *D. caninum*.
5 „ „ *A. mystax* and *D. caninum*.
2 „ „ *D. caninum* and *T. serrata*.
2 „ „ *A. mystax*, *D. caninum* and *T. serrata*.

The numbers of *A. mystax* varied from 1 to 113 per dog.
„ „ „ *D. caninum* „ „ 1 to 102 „ „
„ „ „ *T. serrata* „ „ 1 to 8 „ „

The total number of each species recovered from the 24 dogs was

A. mystax 296.
D. caninum 312.
T. serrata 17.

The following table contains details regarding the numbers and species of worms found in each of the dogs examined:—

No. of dog	Species of worm	No. of worms found
1	*A. mystax*	4
2	*D. caninum*	1
	T. serrata	3
3	*A. mystax*	2
	D. caninum	2
	T. serrata	1
4	*A. mystax*	5
5	*A. mystax*	1
6	*A. mystax*	48
7	*A. mystax*	4
8	*D. caninum*	7
9	*D. caninum*	20
	T. serrata	8
10	*D. caninum*	7
	A. mystax	2
11	*A. mystax*	4
	D. caninum	1
12	*A. mystax*	14
13	*A. mystax*	3
14	*D. caninum*	1
15	*D. caninum*	82
16	*A. mystax*	2
17	*D. caninum*	30
	A. mystax	6
18	*D. caninum*	1
	A. mystax	45
	T. serrata	5
19	*D. caninum*	1
20	*D. caninum*	53
	A. mystax	7
21	*A. mystax*	5
22	*A. mystax*	31
23	*A. mystax*	113
	D. caninum	4
24	*D. caninum*	102